Cellular Immunity Foundation

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September 9, 2000

Stephen C. Groft, Pharm.D.
Executive Director
White House Commission on Complementary
and Alternative Medicine Policy
6701 Rockledge Drive, Suite 1010, MSC 7707
Bethesda, MD 20817

Dear Dr. Groft:

Dr. Margulis and I were pleased that you were able to spend a few minutes with us after the session yesterday morning in San Francisco. As you know, we made a presentation on Topical Immune Modulation (TIM) as a treatment for asthma. I said that although we think the treatment of asthma is important, our main focus has been on the use of TIM in treating HIV disease. I offered to send you some literature on that subject, which is the purpose of this letter.

I also want to report a call I had from Dr. Margulis this morning. He asked me to remind you that because TIM is a preventive for asthma and because asthma, particularly in children, can be fatal, in determining the value of TIM one needs not only to consider the very large cost of treating asthma in emergency rooms and as in-patients but also because lives our saved by preventing an asthmatic episode.

A number of documents about TIM follow this letter. (TIM is sometimes referred to as DNCB therapy because DNCB is the immune modulator.) Although our primary interest has been in using TIM for HIV disease, there have been indications for some time that there are a number of other systemic, intracellular infections and cellular immunity disorders, including asthma, that are likely candidates for TIM. I comment on the documents later in this letter.

Before the comments, however, a look at the larger picture may be useful. TIM is a treatment designed for systemic intracellular infections and cellular immunity disorders. A number of such infections and disorders in addition to HIV infection have been treated or are likely to be treatable with TIM. (Numbers following the comments are keyed to the documents.)

- Human Papillomavirus (cervical warts). TIM has been shown to eliminate the virus in a pilot study (see 6).
- Leprosy. A phase I trial of TIM is being planned (see 3).
- Lupus. TIM was tried once and appeared to work (see 3).
- Malaria. A beneficial side effect of the TIM trial in Brazil was that parasitic infections were reduced (see 4). As you know, malaria runs almost neck and neck with HIV disease and often accompanies HIV disease in sub-Saharan Africa.

- Chronic fatigue syndrome. A pilot study of TIM with 30 patients showed improvements (see 3).
- Atopic dermatitis. A pilot study has demonstrated the effectiveness of TIM with this skin version of asthma (see 10).
- Two studies have shown that there is a high inverse correlation between the degree of response to DNCB and asthma. We speculate that increasing the response by repeated use of DNCB would reduce or eliminate asthma (See 9).
- Tuberculosis associated with HIV disease. We believe that when cell-mediated immunity (CMI) is increased through the use of TIM, that increase will result in TB drugs that had failed to work again become effective.

TIM is obviously not a cure-all. Nevertheless, it appears to have the potential of being effective for a number of systemic, intracellular infections and cellular immunity disorders. As you know, many such infections lead to very serious world problems. With DNCB being nearly cost-free, there are few avenues for medical exploration that appear to offer a greater prospect than TIM for improving global health. Only pilot studies and clinical trials for each infection or immune disorder will provide an answer.

The documents being transmitted are the following:

1. Purpose of our Foundation.

2. CVs of our Medical Advisory Board and of me.

3. A paper on the medical uses of DNCB during the past 70 years. It shows how contact sensitization with DNCB results in delayed-type hypersensitivity that in turn causes an increase in CMI. It also summarizes pilot studies and clinical trials of TIM, with emphasis on the treatment of HIV disease.

4. A paper on the first two years of a clinical trial of TIM at a large charity hospital in Porto Alegre, Brazil, which trial started in 1995.
   - The trial was a controlled but not randomized or double-blinded. Patients received either TIM or no treatment. The results were first presented at the Vancouver Conference.
   - An abstract of a follow-up paper, presented at the Geneva Conference, is also included.
   - TIM has been used with success at the hospital for six years.

5. An abstract of the first year of a two-year clinical trial of DNCB at the Southwestern Medical Center of the University of Texas, funded by NIH.
   - All patients had been and continued to be on antiretrovirals, including a PI.
   - The trial was a controlled, randomized, double-blinded trial with three arms: DNCB, SLS (another skin irritant), and acetone.
   - As the abstract shows, patients in the DNCB arm did substantially better than those in the other arms.

- When a paper covering the two years was submitted for publication, reviewers suggested that the trial should be extended to include a crossover of DNCB and SLS.
- A crossover was then initiated and the paper with the results of the crossover is now being readied for resubmission.
- Dr. Cruz, the principal author, has told us that the crossover reinforces the benefits of DNCB shown in the abstract.

6. An abstract about the use of DNCB in treating human Papillomavirus (cervical warts).

- This pilot study shows that TIM was very effective in treating this important cause of cervical cancer.
- The principal author, Dr. Salvo, has told us that the treatment has been used successfully during the nine years since the study was completed.

7. Letters in connection with an IND for the treatment of HIV disease solely with a DNCB patch, which was to be conducted in Hawaii.

- This IND was approved in February 1996, about four years after approval was first requested.
- When approval was first requested, few ARV drugs were available and there was considerable interest in DNCB in the medical community.
- However, by the time the FDA approved the trial, there were many ARVs, including the newly offered PIs, which made it impossible to recruit participants. The trial was finally abandoned.
- For this reason, the trial at the University of Texas, initially conceived as for TIM alone, included ARVs.
- This points up a major problem in testing TIM by itself. By now, a trial of TIM by itself that is open to all who are infected would probably not be approved in an industrialized country. Depriving them of the standard of care would be considered unethical.
- There is, however, the possibility of a trial of TIM alone involving people that have abandoned HAART, estimated to be roughly a third of those who have used HAART.
- As to lesser developed countries, we think it would be unethical to reject a clinical trial of TIM by itself in countries where the standard of care is in fact no treatment at all, given that TIM is affordable everywhere. We understand, however, concern by African and other developing countries about using their citizens as guinea pigs. We have discussed this matter with many ambassadors, Ministers of Health, and grass-roots AIDS workers from African nations, several of whom have invited us to come to their countries to help them organize clinical trials or treatment programs (which assistance we are unable to afford). We believe that if there are to be clinical trials of TIM in those countries, it is essential that Western involvement be limited to technical assistance.

8. An unpublished article about treating HIV disease with DNCB.

- This article is an expanded and less formal version of item 3 above. Dr. Raphael Stricker, our Medical Director, and I prepared it.
- Since it was prepared a year ago, it does not reflect some recent developments, including the abandonment of the Hawaiian IND.

9. Two papers on the correlation of asthma and sensitivity to DNCB. (Not included; these accompanied the letter we delivered to the commissioners.)

10. A paper on the treatment of atopic dermatitis. (Not included; this paper accompanied the letter we delivered to the commissioners.)

11. A 1993 paper on a pilot study of DNCB therapy for HIV disease that first showed the effect of topical DNCB application on HIV RNA levels in serum.

couldn't locate - will send later

12. A 1995 paper concerning determining quantitation of HIV1 RNA levels in plasma, in the discussion of which there is reference to the results of DNCB on those levels.

13. A 1997 paper that confirmed that there is a decrease in viral load associated with DNCB therapy in HIV disease. In particular, plasma HIV RNA levels decreased by one-half to more than one log in each DNCB treated subject and decreased significantly in the control group.

14. My letter to Lancet regarding TIM.

15. An unpublished paper that we inspired about a very small pilot study in which DNCB was applied to pregnant mice to see if there were any adverse effects on either the mother or the offspring. Not only were no adverse effects observed but also the weight of newborns at birth was about 10% above average. We requested this study as the first step in determining the potential for TIM in preventing perinatal transmission

16. A letter from the former Director of Bioassay at the National Cancer Institute confirming that DNCB is neither carcinogenic nor mutagenic.

17. A paper showing that prior immune dysfunction predisposes homosexual men to HIV infection.

As you can see, TIM appears to have an enormous potential for improving global health. On top of all its potential benefits, it is for all practical purposes free and it does not require a sophisticated medical delivery system to be administered, as HAART does.

The biggest barrier to the use of TIM, in our opinion, is that DNCB, which can't be patented and is produced in industrial chemical quantities, is so cheap. We have talked to major drug companies about distributing DNCB and they all say we must be crazy to think that they would advocate a very inexpensive treatment that competes with their HAART drugs. Regretfully, the movers and shakers in medical research are not much help

either, because they receive big bucks from the drug companies for researching and testing HAART drugs, not to mention lecture fees and the like. (In this connection, I recall the remark of two UCSF faculty members yesterday when they said that universities were the ideal place for CAM research, in part because the institutions and their faculties don't have conflicts of interest. A member of the same faculty recently published a study showing that there was widespread conflict of interest!)

Because of the frauds and misrepresentations occasionally arising in connection with CAM (in honesty, from conventional medicine as well, but many tend to ignore that) we have been somewhat reluctant to be identified with CAM. More recently, however, given the frequency with which we have been rebuffed by convention, we decided that we should take the leap, which is why we showed up yesterday. In short, we think there is a tremendous potential for TIM and that the Commission's support could make a major difference, not just with asthma but also with HIV disease and the other diseases discussed above.

When we spoke with you after the meeting, Dr. Margulis referred to someone at NIH who met with a group of African AIDS workers (whom we later entertained). Her name is Sudha Srinivasan, Ph.D. and she is Program Officer for Africa and the Middle East. She was said to have told them that she had on the order of a billion to spend on AIDS projects related to Africa and the Middle East. I will be e-mailing her soon to follow up on that lead; I will send you a blind copy.

Dr. Margulis also left with you an e-mail regarding IL-2, a treatment for HIV disease advocated by Fauci, on which hundreds of millions are proposed to be spent or are being spent on clinical trials. I got that because I am on the e-mail distribution list of Billi Goldberg. In our minds she is on of the most profound thinkers regarding HIV disease. Billi reads everything in the literature, exchanges correspondence with authors of important articles, writes commentaries on the treatment of AIDS, and distributes them by e-mail. I will forward some of his recent distributions to you and will continue to do so until you let me know if you would like to be on is list. We will then arrange that.

Any suggestions you have on any of the above would be most appreciated.

Sincerely,

Richard De Lancie

Richard De Lancie, President

Copy to Silvio Margulis, M.D.

Cellular Immunity Foundation

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Purpose of Cellular Immunity Foundation (CIF)

CIF was formed in 1997 for the purpose of advocating the treatment of chronic systemic diseases by increasing cellular immunity, with emphasis on HIV/AIDS. Currently, the most effective means of accomplishing this are topical dinitrochlorobenzene and methyl donor immunotherapy. The result of these therapies is to increase cellular immune responses against HIV and other intracellular infections. The therapies are thoroughly grounded in scientific literature and DNCB therapy has been clinically tested for 13 years.

Antiretroviral drugs, the standard treatment in the industrialized world, do not work for about one third of those who have access to them, have very severe side effects, and, at about $15,000 per year, can't be afforded for over 95% of those infected worldwide. Our therapies cost less than 1% of antiretroviral therapy, so that they can be afforded throughout the world. Furthermore, they do not have any of the medical limitations of antiretroviral therapy.

CIF's activities are grossly restricted by lack of administrative support and of funds to expand contact with AIDS workers worldwide. CIF is now run entirely on a volunteer basis, with two people, a retired CEO of a listed company and an Argentine physician, its Director of Clinical Trials, working essentially full time. It operates out of the home office of the former.

Competence of CIF

CIF has a Medical Advisory Board that includes physicians who have carried on most of the research, pilot studies, and clinical trials of our therapies. It includes three medical school faculty members (a former Department Chairman at a leading school, the present Department Chairman at another leading school, and the physician who initiated research on DNCB 13 years ago), the Director of Sexually Transmitted Diseases at a large Brazilian charity hospital, and the Medical Director of CIF, who has published widely about DNCB therapy.

Specific Objectives of CIF

- To establish and maintain a web site to which AIDS workers around the world can refer for technical information about our therapies.
- To increase contacts with AIDS workers around the world. (Contacts with over 400 of them have already been made.)
- To encourage other organizations to fund clinical trials of our therapies in lesser-developed countries.
- To fund administrative and fundraising support to support the foregoing.

Results expected from achieving the objectives

- Increasing awareness, especially in the lesser-developed countries, that there are effective therapies for HIV disease that are better than antiretroviral therapy and that can be afforded worldwide.
- Encouraging clinical trials of our therapies in lesser-developed countries.
- Raising of funds to permit further expansion of CIF's activities, including the development of an international network of foundations focussed on our therapies.
- Building a theoretical basis for the use of our therapies to prevent (as well as treat) HIV disease.
- Extending the use of our therapies to other systemic diseases, such as cancer, lupus, atopic disease, chronic fatigue syndrome, leprosy, and certain forms of tuberculosis.

Cellular Immunity Foundation

Medical Advisory Board

Raphael B. Stricker, M.D., Chairman.

Dr Stricker received his medical degree and training in Internal Medicine at Columbia University. Hie did subspecialty training in Hematology/ Oncology at the University of California, San Francisco, and supplemental training in Immunology and Immunotherapy at California Pacific Medical Center in San Francisco. He is a member of the American Society of Hematology and the Clinical Immunology Society. He has been an Assistant Professor of Medicine at the University of California, San Francisco, and Associate Medical Director, Division of Immunotherapy at the California Pacific Medical Center. Dr. Stricker has been doing research on HIV disease for 14 years. He has had many papers on DNCB published in medical journals. His clinical practice includes many people with HIV disease. Dr. Stricker is Medical Director of our Foundation.

Jean C. Borderon, M.D.

Dr. Borderon received his medical degree from the University of Paris, where he became an instructor in the Parasitology and Tropical Medicine Department. There he was certified in Pediatrics, Hematology, Parasitology, Microbiology, Serology, and Hygiene, and later, at the Pasteur Institute in Paris, in Medical Mycology and Epidemiology. He then became a fellow and lecturer in Pediatrics, Microbiology, and Hygiene at the University School of Medicine, where he formed a microbiology laboratory and the Pediatric Infectious Disease Unit at the Pediatric Hospital. He has lectured regularly, including seminars to French speaking infectious disease specialists from African nations, many of whom he visits during frequent trips to Africa. He has also participated in many international congresses and seminars. His publications have included reports on his clinical trials on infectious diseases, as well as on his research on pediatric infectious diseases and infection control. He has contributed to the growth of knowledge about infectious diseases, including HIV disease, by coordinating annual conferences and directing an annual certification course on pediatric infectious diseases at the University of Tours, again with emphasis on HIV disease.

Ponciano D. Cruz, Jr., M.D.

Dr. Cruz is Chairman of the Department of Dermatology at the Southwestern Medical Center of the University of Texas at Dallas. He is the Principal Investigator of a large, two-year, double blind clinical trial of DNCB in Dallas, funded by NIH. In this trial, DNCB is being used together with combination antiretroviral therapy. Dr Cruz presented the results of the first year of the trial at a meeting in Cologne in May of 1998, where he showed that as compared with patients receiving antiretroviral therapy alone those who were also treated with DNCB "showed steep increments in CD4 and CD8 cell counts" which "was paralleled by significant reduction of plasma load and by improvement in patients' assessment of overall health, skin health, and ability to care for self".

William L. Epstein, M.D.

Dr. Epstein received his medical degree from the University of California, San Francisco, where he served his internship. Subsequently, he was a Resident at the Hospital of the University of Pennsylvania. The American Board of Dermatology certified him in 1956. After he completed his residency, he became a member of the faculty in the Department of Dermatology at the University of California, San Francisco, where he is now a Professor. He was Chairman of the Department for 11 years. Dr. Epstein has authored a number of papers on DNCB and is known especially for his development of a patch for applying DNCB to the skin. (The method of application first used, which is still in use, is to apply a solution of DNCB in acetone directly to the skin.) Dr. Epstein has been awarded many honors, including the highest civilian award by the government of Japan.

Silvio B. Margulis, M.D.

Dr Margulis received his medical degree at the School of Medicine, University of Buenos Aires. Residencies at Tufts Medical School, Harvard Medical School, concluded at Children's Hospital in San Francisco. American Board of Pediatrics, certified in 1964. For the past twelve years Dr. Margulis principal interest has been HIV disease for all affected populations. He attended the last four World AIDS Conferences and meetings elsewhere. He chaired symposiums at the last two World AIDS Conferences and was the first presenter of data regarding a clinical trial of DNCB therapy for HIV disease from a third-world country. Results of the first two years of that trial have been reported in the medical literature, with Dr. Margulis as one of the authors. The trial is still underway in Brazil. He is doing research on the feasibility of cellular immunity treatments not only as the basic treatment for HIV/AIDS but also as a prophylaxis. Dr. Margulis is Director of Clinical Trials for our Foundation.

L. Bruce Mills, M.D.

Dr Mills received his medical degree at the School of Medicine, Stanford University, subsequently receiving certification as a Dermatologist. In 1980, while in private practice, he treated HIV infected patients whose symptoms included Kaposi's Sarcoma. He knew of the success in treating other skin conditions with DNCB and tried it on his patients, with notable success. He published a paper on that finding and subsequently has been the author of a number of other papers about treating HIV disease with DNCB. Dr Mills is now Director of the Dermatology Clinic at the University of Hawaii. He is also Principal Investigator of a clinical trial of DNCB (as the sole treatment for HIV disease) being carried out under an Investigational New Drug Application (IND) of the FDA. The trial has been underway for two years.

Ari Traub, M.D.

Dr Traub received his medical degree from the Medical School of the Federal University of Rio Grande da Sul (UFRGS) in Brazil. He subsequently received training as a Dermatologist from UFRGS and did research on Syphilis, Leishmania, and AIDS at UFRGS and at the Brazilian Ministry of Health. He is now Chief of the Sexually Transmitted Disease Unit of Irmandade da Santa Casa Misericordia, P.A. (ISCMPA), a charity hospital in Porto Alegre, Brazil. Because of the nature of his work at ISCMPA he has attended many meetings concerned with HIV disease, including World AIDS Conferences. As a result he became interested in DNCB and was able to organize a clinical trial of DNCB at ISCMPA, which is now in its fifth

year. He treats HIV infected patients with DNCB by itself with very encouraging results, particularly a substantial reduction of opportunistic infections, the principal cause of death from AIDS. Because ISCMPA is a charity hospital, his patients are quite similar to those who could be treated for HIV disease in Africa, Asia, and in other parts of South America. A paper describing the results of the first two years of Dr Traub's clinical trial was presented at the 11th World AIDS Conference in Vancouver in 1996 and another paper with subsequent results was presented at the 12th World AIDS Conference in Geneva in June, 1998.

Aurelio Salvo, MD

Dr. Salvo received his medical degree from the University of Chile, followed by Residencies there in Pediatrics and Dermatology. Upon completion of his Residencies he became a Dermatologist at the Dr. Sotero del Rio Hospital, principal hospital of Servico de Salud Metropolitano Sur Oriente (SSMSO). After joining the hospital he organized a Sexually Transmitted Disease (STD) Clinic there. He is now Chief of both the STD Clinic and the STD Prevention and Control Program at the hospital as well as a Professor of Medicine at the University of Chile. Dr. Salvo is a member of the STD Committee of the Chilean Ministry of Health. He is also member of the Chilean Dermatology and Venereology Society and has been certified as a Dermatologist and Venereologist (STDs) by CONACEM. He has undertaken research, made presentations to professional societies, and published many papers, mainly on congenital syphilis, gonorrhea, treatment of women's cervical HPV infections with DNCB, bacillary angiomatosis and eosinophilic pustular folliculitis in HIV patients, and HIV viral load. Dr. Salvo is a member of the Scientific Board of the 10th Latino American STD Congress and of ULACETS for both its last meeting and the next one in Brazil. (Of particular interest to CIF is Dr. Salvo's research and clinical experience with the use of cellular immunotherapy, using DNCB as the immunostimulator in cervical papilloma virus, showing that the use of that therapy is not restricted to HIV disease.)

John H. Weisburger, PhD, MD (hc)

Dr. Weisburger received his PhD in organic chemistry with emphasis on cancer research. He is Research Professor of Pathology at New York Medical College, Director Emeritus of the Naylor Dana Institute for Disease Prevention, and Senior Member of the American Health Foundation. For a period of many years prior to those affiliations, he was with the National Cancer Institute, initially as a Postdoctoral Fellow and ultimately as Director of the Bioassay Segment of its Carcinogenesis programs. He published over 500 papers reflecting his research, many on carcinogenesis and mutagenesis. (His work is of particular significance to CIF, since he demonstrated that DNCB is neither carcinogenic nor mutagenic.) The Public Health Service has honored Dr. Weisburger in many ways for his work, including the National Defense Service and Meritorious Service Medals. Other honors include a MD (hc) from the University of Umea in Sweden and Distinguished and Meritorious Service awards from many professional societies.

Richard De Lancie

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RESUME

HIGHER EDUCATION University of California (Berkeley).1940, Geology, AB1948-50, Ph.D. candidate, Mathematical Statistics. (Recalled by Navy; did not return.)

BUSINESS AND PROFESSIONAL ACTIVITIES

1940-41, Geologist, Shell Oil Company.

1942-46, Naval Officer, Ensign to Lt. Commander. Duties included:
- Photo Intelligence Officer, 1st Marine Division, Guadalcanal.
- Chief Instructor, Naval Photo Intelligence School.
- C.O., largest photo intelligence center in Pacific.
- Chief of Photo Intelligence, U.S. Strategic Bombing Survey.

1946-48, Asst. Mgr., Economic Planning Dept., Pan American Airways.

1951-52, Director of Operations, Naval Photo Intelligence Center.

1953-54, Operations Analyst, U.S. Air Force.

1954-73 URS Corporation. NY Stock Exch. listed: software, architecture, and engineering.
- 1954-57, Operations Analyst.
- 1957-71, CEO. Took company public. Acquired four companies.
- 1971-73, Vice Chairman.

1972-73, Provident Enterprises, founder (provide capital for minority businesses). Chairman when acquired by Opportunity Capital.

1972-83 & 1995- , Opportunity Capital Corporation (capital for minority businesses) 1972-83, Director 1974-79, Chairman of the Board 1995- , Director1973-76, Chairman, Remote Computing Corporation.1976-78, General Partner, Dever Associates, Options Market maker, Pacific Stock Exchange.

1979- , Occasional consultant, financing of small businesses.

OTHER ACTIVITIES

1959-77, American Civil Liberties Union of Northern California.
- 1959-77, Director.
- 1972-77, Chairman of the Board.

1963-67, Founder and President, Fair Housing Council of San Mateo,

1967-69, Director, Actors' Workshop

1971-73, Director, American Conservatory Theater Association.

1993- , Volunteer, Shanti (assisting people with HIV disease).

1997- , Founder and President, Cellular Immunity Foundation. (Treatment of HIV disease.)

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letters

Topical immune modulation (TIM): a novel approach to the immunotherapy of systemic disease

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Abstract

In this article, we present the concept of topical immune modulation, or TIM. TIM is based on the observation that skin contact sensitizing agents such as poison ivy, poison oak and dinitrochlorobenzene (DNCB) are potent stimulants of the cellular immune system that combats viruses and other pathogens. We discuss the evolution of DNCB as a therapeutic modality in the acquired immunodeficiency syndrome (AIDS) and we explore the mechanism by which DNCB directs the immune response. The potential use of topical immune modulators in autoimmune disease and vaccine development is also delineated. TIM represents a novel approach to immunotherapy that should have widespread application for immunologic diseases. © 1997 Elsevier Science B.V.

Keywords: Topical immune modulation; Immunotherapy; Dinitrochlorobenzene; HIV; AIDS

1. Introduction

Progress in immunology has evolved in large part from our understanding of the humoral and cellular branches of the human immune system. Over the past century, strategies to enhance the humoral immune response have developed logically and successfully, with improving vaccine technologies and refined immunoglobulin therapies. In contrast, attempts to boost the cellular immune response have been largely unsuccessful. This lack of success is due in part to the forbidding complexity of the cellular network and in part to our limited understanding of key cells in the network, such as epidermal Langerhans cells, dendritic cells and other antigen presenting cells. Lack of success in boosting the cellular immune response has been underscored by the spread of the acquired immune deficiency syndrome (AIDS), a disease characterized by cellular immunodeficiency induced by the human immunodeficiency virus (HIV). The list of therapeutic failures in this disease attests to our inability to reverse HIV-induced damage to the cellular immune system.

Table 1
Contact sensitizing agents used in laboratory animals and humans

Compound	Mutagenic	Carcinogenic
Dinitrochlorobenzene (DNCB)	Yes	No
Dinitrofluorobenzene (DNFB)	Yes	Yes
Diphencyprone (DPCP)	No	Unknown
Oxazolone (OXA)	Unknown	Unknown
Paraphenylenediamine (PDA)	Yes	Unknown
Squaric acid dibutylester (SADBE)	No	Unknown
Urushiol (poison ivy, poison oak)	Unknown	Unknown

Mutagenicity was determined by in vitro testing (Ames assay), while carcinogenicity was determined by in vivo testing (animal and/or human studies).

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Topical immune modulation, or TIM, is a novel concept based on an old observation that topical contact sensitizing agents such as poison ivy, poison oak and dinitrochlorobenzene (DNCB) are potent stimulants of the cellular immune response. As outlined below, DNCB and other skin contact sensitizers (Table 1) have been used by researchers and clinicians for many years as diagnostic tools to evaluate cellular immune function. The concept of using DNCB as a therapeutic modality was galvanized by the novelty and severity of the AIDS epidemic. This therapeutic approach is supported by recent advances in our understanding of HIV and the immune havoc that it wreaks.

2. DNCB background

First, consider some of the reasons why DNCB, a simple photochemical, is a favorite experimental skin contact sensitizer for use in man. In the late 1920s, DNCB was recognized as an occupational contact sensitizer [1] and then used for experimental cutaneous sensitization [2]. In the 1930–40s, Chase and Landsteiner performed classic studies showing that DNCB and other chemicals could sensitize the skin of guinea pigs and that this cutaneous sensitivity could be transferred by peritoneal leukocytes [3]. The skin reactivity was labelled delayed-type hypersensitivity (DTH), a lymphocyte-driven condition that reflects cell-mediated immune function. During this time, Sulzberger et al. used DNCB in patients with various skin diseases to see if some were more susceptible to the compound than others [4]. In the 1950s, Epstein and Kligman explored complex DTH testing in otherwise healthy volunteers [5], setting the stage for investigation of the cellular immune status of all types of patients. Similar DTH studies were carried out by a number of workers in the 1960s [6]. However in 1972, a landmark paper by Catalano et al. [7] described a simple standardized procedure for immunologic evaluation using cutaneous DNCB sensitization. This method opened the door for DNCB testing in many immunodeficiency diseases over the next 10–15 years.

At the same time, dermatologists were exploring the use of DNCB as a treatment for cutaneous diseases that might be subject to immune modulation, such as recalcitrant warts, various forms of alopecia (baldness) and even metastatic melanoma [8]. In each of these instances there were some reports in which the warts disappeared and the hair grew back, but often when DNCB was stopped the warts grew back and the hair fell out again. Even metastatic melanoma lesions would regress (and sometimes lesions that were not directly treated with DNCB would disappear), but often the overall course of the disease was not altered by the DNCB regimens used at the time. Furthermore in the late 1970s, DNCB was found to have mutagenic activity in the Ames test [9], although it was not a carcinogen (Table 1). This negative information generated extensive discussion in the medical literature [10] and the upshot of the controversy was to discourage many dermatologists from using DNCB.

3. The AIDS epidemic

The AIDS epidemic arrived in the early 1980s with reports of cellular immunodeficiency in homosexual men, intravenous drug users and hemophiliacs. Dr L. Bruce Mills, a dermatologist who had experience with DNCB skin testing, decided to treat some of his AIDS patients with the topical compound. His clinical results were encouraging and were published in 1986 [11], but Mills' small study was uncontrolled and had collected a minimal amount of immunologic data. Nevertheless, AIDS activists realized that this inexpensive, readily available treatment at least offered a means to combat the deadly viral infection. Over the next few years, DNCB would become the most widely used alternative treatment for HIV disease. The initial enthusiasm for DNCB therapy failed to generate support in the medical community, however, and the compound was generally employed in an uncontrolled and unmonitored fashion. Excessive application of the compound resulted in toxic skin reactions that were well publicized [12].

By 1988, it was decided that a DNCB monitoring study had to be performed. A small trial was organized by a research group in San Francisco with the approval of the Food and Drug Administration (FDA). Unfortunately, this study was never initiated for a variety of reasons. AIDS researchers were not convinced that DNCB worked and by this time they had other antiviral drugs to study, such as zidovudine (AZT). Furthermore, since DNCB is inexpensive and cannot be patented, no pharmaceutical company would provide the financial assistance to ensure that all of the immunologic testing required for the trial would be carried out. The compound remained untested.

4. DNCB and AIDS

A DNCB pilot study was finally organized in 1990. Topical DNCB was applied by the study subjects on a weekly basis according to a standardized clinical protocol [13]. The study involved monitoring of T-cell subsets and viral load in blood samples from 24 patients with early-stage HIV disease who were sensitized with DNCB. These blood samples provided immunologic evidence that DNCB might be helpful for the following reasons: studies of lymphocyte subsets had shown that

Table 2
Viral load in HIV-infected patients treated with dinitrochlorobenzene (DNCB)

HIV RNA (copies/ml)	n	Pre-DNCB (mean ± SD)	On DNCB (6 months) (mean ± SD)
RNA < 10 000	5	4220 ± 894*	6840 ± 1444*
RNA > 10 000	6	61 733 ± 22 007**	21 400 ± 7273**

Serum HIV RNA levels were quantitated using a reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Note that patients with low HIV RNA levels (< 10 000 copies/ml) continued to have a low viral load while using DNCB. In contrast, patients with elevated HIV RNA levels (> 10 000 copies/ml) had a significant decrease in viral load on DNCB treatment. *n*, number of patients; SD, standard deviation.
* P = not significant; ** $P = 0.014$.

elevated cytotoxic CD8 T-cells were more important than CD4 T-cell levels in prognosticating survival in HIV disease [14,15]. Other studies had suggested that if natural killer (NK) cells stayed elevated, the patients did well [16]. This immunologic profile is what was seen in the group treated with DNCB. Even more important, as shown in Table 2, DNCB-sensitized patients with an increased viral burden showed marked decreases in serum HIV RNA as detected by a prototype quantitative polymerase chain reaction (PCR) assay [13]. This finding was recently confirmed in a prospective controlled DNCB trial using a commercial HIV RNA PCR test [17]. Thus patients who were sensitized to DNCB had a diminished viral load and this effect may have contributed to their continued good health.

Subsequently a follow-up report on the pilot study patients was published [18]. The cohort of subjects was divided into those who were compliant, meaning that they applied the DNCB treatment regularly for the full length of the study (an average of more than 2 years) and those who were non-compliant, meaning that after about 11 months with stable disease they stopped treatment but continued to be monitored. The comparative results are shown in Tables 3 and 4.

Table 3
Lymphocyte subsets in compliant versus non-compliant DNCB-treated subjects

Lymphocytes (cells/μl)	n	Pre-DNCB (mean ± SD)	Follow-up (mean ± SD)
Cytotoxic CD8+ (CD8+CD57+)			
Compliant	13	285 ± 274*	343 ± 180*+
Non-compliant	11	308 ± 257*	304 ± 256*+
Activated CD8+ (CD8+DR+)			
Compliant	12	399 ± 322*	502 ± 390*+
Non-compliant	10	497 ± 289*	407 ± 376*+
Natural killer (CD56+)			
Compliant	13	108 ± 62^*	168 ± 74^#
Non-compliant	11	126 ± 66^^*	70 ± 31^^#

Compliant patients had a significant increase in activated cytotoxic CD8 T-cells and natural killer (NK) cells.
* P = not significant; + $P = 0.014$; ^ $P = 0.002$; ^^ $P = 0.04$; # $P = 0.006$.

The compliant group showed significant increases in activated cytotoxic CD8 T-cells and NK cells compared to the non-compliant group (Table 3). More important, the clinical outcome in the compliant group was clearly superior, as shown in Table 4. Of the 13 compliant patients, two developed an AIDS-defining illness (localized Kaposi's sarcoma) and none of these patients died. In contrast, among the 11 non-compliant subjects, five patients developed AIDS and four of the patients died.

Independent confirmation of this study has recently been obtained. Traub and Margulis collected clinical and immunologic data on HIV-infected patients treated with topical DNCB in Brazil [19]. After 21 months of treatment, the 19 patients enrolled in the study had significantly increased CD4 and CD8 T-cell counts. In this population, that normally had numerous parasitic infections, a significant decrease in parasitosis was seen in the DNCB-treated individuals during the study period. Finally, follow-up was obtained on HIV-infected patients initially treated with topical DNCB as a result of the study published by Mills in 1986. The ten patients from this cohort included three long-term AIDS survivors and all were doing well clinically up to 7 years after starting regular DNCB application [20]. Thus in both short-term and long-term studies, DNCB therapy appears to have a beneficial clinical and immunologic effect in HIV disease.

Even more recently, the first DNCB trial in immunocompromised monkeys was performed in Sweden. A group of nine cynomolgus macaque monkeys was infected with a strain of simian immunodeficiency virus (SIV). Five of the monkeys were then treated with weekly topical DNCB applications, while four monkeys served as controls. Over the first 12 months of the study, all of the monkeys remained healthy. However by 16 months, three of the four control monkeys had died of lymphoma. In contrast, the five DNCB-treated macaques remained in excellent health. (S. Eriksson, personal communication). Although this surprising observation requires confirmation in larger controlled trials, it appears that the SIV model may be useful for future immunologic studies of contact sensitizing agents in humans.

3-4

Table 4
Clinical status of compliant versus non-compliant subjects

Subjects	n	On AZT	Mean follow-up (months)	Months on DNCB (range)	Progression to AIDS (%)	Died (%)
Compliant	13	3	28.6	28.6 (14–44)	2 (15)[a]	0 (0)
Non-compliant	11	8	28.9	10.9 (6–24)	5 (45)[b]	4 (36)

A total of 15% of compliant patients progressed to AIDS and none died. In contrast, 45% of non-compliant patients developed AIDS and 36% died.

[a] 2% Kaposi's sarcoma (in remission).

[b] 3% *Pneumocystis carinii* pneumonia, 2% Kaposi's sarcoma (progressive).

5. How does DNCB work?

A major question is: what is the mechanism of DNCB activity, based on evolving concepts of the complex interactions within the cellular immune system? It is known, for example, that the Langerhans cells of the epidermis and dendritic cells throughout the body are heavily infected with HIV and that the infected cells have an impaired antigen-presenting capacity [21–23]. Exposure to DNCB may correct the dysregulation that develops between the infected antigen-presenting cells and T-cells, leading to control of HIV replication in various organ systems. This immune 're-regulation' would result in a lowered viral load, as reflected by the blood HIV RNA levels (Table 2).

Another possibility is that repeated exposure to DNCB amplifies the Th1 CD4 T-cell subset that regulates cell-mediated immunity, with a resultant downregulation of the Th2 CD4 T-cell subset that stimulates humoral immunity [24,25]. Consequently the predominant cytokines produced in lymph nodes and other parts of the body would take on a Th1 profile of cytotoxic antiviral activity and would antagonize the release of Th2 cytokines responsible for the ineffective humoral response seen in HIV disease. Upregulation of the Th1 response by DNCB could therefore reverse the pathological 'Th1/Th2 switch' described in HIV patients [26], with consequent mobilization of cytotoxic CD8 T-cells and NK cells (Table 3). Since the immune dysregulation of AIDS results in diminished cytotoxic activity against HIV, this cellular immune-boosting effect of DNCB should help to keep progression of the disease in check. Thus DNCB promotes the type of cell function and cytokine production that may be necessary for survival in AIDS [26,27].

6. Other applications of TIM

From the foregoing discussion, it seems logical that a topical immune modulator such as DNCB would be useful in other immune-mediated diseases. Three diseases have been targeted so far. Chronic fatigue syndrome (CFS) is a debilitating multisystem disease of unknown etiology that is probably related to infection by a virus (or group of viruses) [28–30]. CFS is characterized by debilitating fatigue, neurologic deficits and brain scan abnormalities [28]. Lymphocyte subset alterations have also been described in this disease [29,30]. Topical DNCB has been used in about 30 patients with CFS (D. Payne, personal communication). The patients had improvement in fatigue, cognitive function and brain scan lesions, although lymphocyte subset changes were not examined. Further study of DNCB in CFS is now in progress.

Two other diseases that may be amenable to TIM are systemic lupus erythematosus (SLE) and leprosy. SLE is a multisystem disease characterized by autoantibody production against a number of cellular antigens [31,32]. This autoimmune response is thought to be due to an overactive Th2 repertoire [32]. Since DNCB appears to downregulate the Th2 response, it is possible that this compound could help to control the autoimmune processes in SLE. Topical DNCB treatment of a single SLE patient has supported this possibility [33], but larger trials are needed to examine the effect of DNCB in SLE patients. Leprosy is a chronic infectious disease characterized by disfiguring skin lesions, T-cell dysfunction and a host of autoimmune phenomena [34,35]. These autoimmune reactions contribute to the pathology of the disease. As in SLE, DNCB application in leprosy should help to diminish the autoimmune response involved in progression of the disease. A controlled trial of DNCB in leprosy is currently in the planning stages in Hawaii (L.B. Mills, personal communication).

In addition to these therapeutic applications, DNCB or related compounds may be useful for vaccine development. Current vaccines promote antibody-mediated (Th2-based) immune responses and there is a great need for vaccines that induce cell-mediated (Th1-based) immunity, especially in the therapy of cancer and AIDS [36–38]. An analogue of DNCB has been used to develop a melanoma vaccine [39] and a similar approach is being considered for an AIDS vaccine. The use of TIM-related technology for these novel vaccines holds great promise for an area of immunology that has made little progress in the last 20 years.

7. Summary

The AIDS epidemic has fostered a novel approach to immunotherapy in the form of DNCB and topical immune modulation (TIM). Many questions remain concerning the optimal dose, dermal reactivity, skin location and frequency of DNCB application necessary to enhance the immune response in HIV disease and other conditions. However, the concept of topical immune modulation in general and the use of DNCB as an immune modulator in particular, opens the door to a new era in which contact sensitizing agents will play an increasingly important role in the treatment of immunologic diseases. Optimizing the use of these low-tech, easily accessible compounds will pose a significant challenge to dermatologists and immunologists in the coming years.

Acknowledgements

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Topical Immune Modulation with Dinitrochlorobenzene in HIV Disease: A Controlled Trial from Brazil

Key Words
Topical immune modulation
Immunotherapy
Dinitrochlorobenzene
HIV
AIDS
Brazil

Abstract

Objective: Despite the rapid spread of human immunodeficiency virus (HIV) in the developing countries of Africa, Asia and Latin America, accessible and affordable antiretroviral therapies have not been developed. Dinitrochlorobenzene (DNCB) is an inexpensive contact sensitizing agent that stimulates cell-mediated immunity when applied to the skin. We have examined the clinical and immunologic effects of topical DNCB therapy in a cohort of indigent patients with HIV disease from Brazil. **Design and Methods:** Thirty-five HIV-infected subjects were divided into a control group that refused DNCB therapy (6 patients) and a treatment group that applied topical DNCB on a weekly basis throughout the study (29 patients). Subjects were monitored for adverse clinical events, progression to AIDS and changes in body weight. CD4 and CD8 T-cell counts were also monitored in both groups. **Results:** Control and treated patients were evenly matched in terms of age, initial clinical status and prior adverse clinical events. The mean follow-up was 19.7 months for the control group and 17.8 months for the DNCB group. Control patients had significantly more adverse clinical events and progression to AIDS during the study than the treatment group ($p=0.002$ and $p=0.013$, respectively). There were no deaths in either group. Control patient weights decreased over the study period while DNCB patient weights increased ($p<0.001$). CD4 and CD8 T-cell counts decreased significantly in the control group and increased in the DNCB group ($p<0.001$ and $p=0.031$, respectively). DNCB therapy was well tolerated. **Conclusions:** Topical DNCB therapy affords a rational, effective and inexpensive treatment approach for HIV disease. DNCB should benefit patients in developing nations with limited access to health care.

Introduction

In recent years, the acquired immune deficiency syndrome (AIDS) has spread rapidly in the developing countries of Africa, Asia and Latin America [1, 2]. It is estimated that by the year 2000, as many as 40 million people with human immunodeficiency virus (HIV) infection, or 90% of the world's cases, will be found in these countries [3]. Efforts to control the spread of AIDS in developing nations have focused primarily on prevention of HIV trans-

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Table 1. Clinical characteristics of study subjects

Subjects	n	M/F	Mean age ± SD, years	On study ± SD, months	Initial clinical status		Adverse clinical events		Progression to AIDS
					asympt./ARC	AIDS	prior to study	during study	
Control	6	4/2	34.3 ± 3.8	19.7 ± 2.8	5/6 (83)	1/6 (17)	3/6 (50)	6/6 (100)*	2/5 (40)**
DNCB	29	15/14	37.0 ± 2.4	17.8 ± 1.3	25/29 (86)	4/29 (14)	12/29 (41)	6/29 (21)*	1/25 (4)**

n = Number of patients; M/F = male/female ratio; SD = standard deviation; asympt. = asymptomatic; ARC = AIDS-related complex. *p = 0.002, **p = 0.013. Figures in parentheses indicate percentages.

mission, since antiretroviral chemotherapy is financially unfeasible for these countries [1–3]. Development of affordable HIV therapies for the bulk of the world's population remains a significant priority, and little progress has been made in this area.

Dinitrochlorobenzene (DNCB) is a simple contact sensitizing agent related to poison ivy and poison oak that has been used in color photography for over 50 years [4]. DNCB is also a potent stimulant of the cellular immune system when applied to the skin. Topical DNCB therapy has been associated with improved clinical and immunologic parameters in HIV-infected patients when the compound is used on a regular basis over a number of years [5–8]. The cost of this therapy is about US$ 0.30 (30 cents) per week.

We undertook a controlled trial of topical DNCB therapy in a cohort of indigent patients from Brazil, a country with limited access to HIV therapies [9–13].

Patients and Methods

The study was performed at a single public hospital in Porto Alegre where patients received free medical care. Inclusion criteria were as follows: (1) HIV seropositivity with CD4 T-cell counts of 100–600/μl and (2) no prior exposure to DNCB. Thirty-five patients (19 men and 16 women) were enrolled in the trial after informed consent had been obtained. The clinical characteristics of the study subjects are shown in table 1. Risk factors for HIV disease included homosexual contact (15 patients), intravenous drug use (9 patients) and heterosexual contact (11 patients). Six patients underwent an initial topical DNCB application but then refused further therapy for cosmetic reasons. These patients continued to be monitored clinically and served as the control group. The remaining 29 patients had weekly applications of topical DNCB throughout the course of the study.

Topical DNCB therapy was carried out as previously described [5], but with several modifications. DNCB in crystal form (>98% pure) was obtained from Kodak Chemicals SA, São Paulo, and dissolved in reagent-grade acetone. Patients were sensitized with a 10% solution of DNCB applied to a 3-cm-square area of skin. Two weeks

Table 2. Infectious complications during the study

Infection	Control subjects (n = 6)		DNCB subjects (n = 29)	
	prior to study	during study	prior to study	during study
Fungus[1]				
Oral	0	1 (17)	0	0
Pulmonary	0	0	0	1 (3)
Vaginal	0	0	0	2 (7)
Bacteria				
Skin[2]	0	1 (17)	0	0
Pulmonary[3]	0	0	1 (3)	0
Intestinal parasites[4]	2 (33)	1 (17)	7 (24)	1 (3)
Syphilis	1 (17)	0	3 (10)	0
Herpes zoster	0	2 (33)	0	2 (7)
Pulmonary tuberculosis	0	0	1 (3)	0
Pneumocystis carinii pneumonia	0	1 (17)	0	0
Total	3 (50)	6 (100)	12 (41)	6 (21)

Figures in parentheses indicate percentages.
n = Number of patients.
1 *Candida* species.
2 *Staphylococcus aureus*.
3 *Klebsiella*.
4 *Ascaris* (1), *Entamoeba* (4), *Giardia* (1), *Strongyloides* (2), *Taenia* (2), unclassified (1).

Table 3. Mean body weight changes in study subjects

Subjects	n	Mean initial weight, kg ± SD	Mean follow-up weight, kg ± SD	Mean change kg ± SD
Control	6	63.8 ± 8.4	60.3 ± 6.6	−3.5 ± 4.4**
DNCB	29	62.0 ± 8.0*	64.9 ± 9.0*	+2.9 ± 2.6**

n = Number of patients; SD = standard deviation. *p < 0.001, **p < 0.001.

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Table 4. Mean CD4 and CD8 T-cell counts in study subjects

Subjects	n	Initial		Follow-up		Mean change	
		CD4 T cells	CD8 T cells	CD4 T cells	CD8 T cells	CD4 T cells	CD8 T cells
Control	6	317±105[a]	902±286[b]	147±55[a]	546±194[b]	−170±94[a]	−356±193[b]
DNCB	29	351±160[c]	865±460	397±166[c]	1,065±651	+46±111[d]	+200±591[e]

T-cell numbers represent cells/μl. n = Number of patients; SD = standard deviation. [a]p=0.007, [b]p=0.007, [c]p=0.035, [d]p<0.001, [e]p=0.031.

after the initial sensitization, therapy was resumed with the application of 2% DNCB solution to a different 3-cm-square area of skin. The 2% DNCB treatment was continued on a weekly basis throughout the study. However, starting at 2 months after the initiation of therapy, each patient received a 10% DNCB booster application once a month instead of the regular 2% application. Excessive skin reactions were treated with cold compresses soaked in camomile tea. All DNCB applications were performed by a single investigator (A.T.) in a standardized fashion using rotating areas of skin on the trunk and extremities.

Patients had blood tests, body weight measurement and fecal parasitic examination performed approximately every 3 months. Blood tests included complete blood count, Venereal Disease Research Laboratory test for syphilis and lymphocyte subset analysis. Analysis of CD4 and CD8 T cells was performed by one central laboratory using flow cytometry, as previously described [5]. To ensure consistency, baseline and follow-up T-cell studies were performed at a time when patients were not acutely ill. Viral load testing was not available at the time of the study.

Antiretroviral chemotherapy and prophylactic antibiotics were not obtainable by the study subjects. Patients were treated for complications of HIV disease as the need arose. Fungal infections were treated with topical or systemic ketoconazole. Syphilis was treated with high-dose penicillin, and parasitic disease was treated with thiabendazole. Patients with herpesvirus infections received acyclovir, and *Pneumocystis carinii* pneumonia was treated with oral trimethoprim/sulfamethoxazole. Pulmonary tuberculosis was treated with isoniazid and streptomycin. No other medications were used in the study.

Statistical analysis was performed using the paired Student t test for intragroup comparisons and the unpaired Student t test for intergroup evaluations. The t test was used because the analysis involved noncategorical data and the variance of the population was unknown [34].

Results

The clinical and immunologic outcomes of the study are shown in tables 1–4. As shown in table 1, the control and DNCB treatment groups were evenly matched in terms of age and initial clinical status. The incidence of adverse clinical events prior to the study was also similar for the two groups (50 vs. 41%, p=n.s.). There was a relatively high incidence of parasitic disease (9/35 patients, 26%) and syphilis (4/35 patients, 11%) prior to the start of the trial. The mean follow-up was similar for the control and DNCB groups (19.7 vs. 17.8 months, p=n.s.). During the study, each of the control patients experienced an adverse clinical event compared to 6/29 DNCB-treated patients (100 vs. 21%, p=0.002). Over the course of the study, 2/5 control patients progressed to AIDS, while only 1/25 treated patients had disease progression (40 vs. 4%, p=0.013). Adverse clinical events included fungal, bacterial, parasitic and herpetic infections, as shown in table 2. There were no deaths in either group.

Changes in patient body weights are shown in table 3. Initial weights were comparable in the control and treatment groups. The mean weight decreased by 3.5 kg in the control group over the study period (p=n.s.). In contrast, the mean weight increased by 2.9 kg in the treatment group (p<0.001). The difference in mean weight change between the two groups was highly significant (p<0.001).

Changes in mean CD4 and CD8 T-cell counts are shown in table 4. Initial CD4 and CD8 T-cell levels were comparable in the control and treatment groups. In the control group, both the CD4 and the CD8 T-cell counts decreased significantly over the course of the study (p=0.007 for both subsets). In contrast, CD4 T cells increased significantly in the DNCB-treated patients (p=0.035). Although CD8 T-cells also increased in these patients, the difference was not statistically significant. However, the change in mean T-cell counts in the control group compared to the treatment group was significant for both CD4 T cells (p<0.001) and CD8 T cells (p=0.031) over the course of the study.

DNCB applications were well tolerated, and none of the DNCB-treated patients discontinued topical therapy. Excessive skin reactions were effectively controlled with the cold camomile compresses, and no infections were seen as a result of these reactions.

Discussion

The current study represents the first controlled trial of topical DNCB therapy in HIV disease. Patients treated with DNCB had a significantly lower incidence of adverse clinical events and significantly less progression to AIDS compared to controls. They also had significant weight gain and increased T-cell counts compared to the control subjects. These findings confirm previous reports of beneficial clinical and immunologic effects of topical DNCB therapy in HIV disease [5–8]. The study also confirmed the safety of standardized DNCB application over a prolonged period of time [15, 16].

Several features of the current study differ significantly from previous DNCB trials [5–8]. First, the current study included the largest group of women ever treated with topical DNCB. This aspect is particularly important for future use of DNCB in developing countries where the majority of HIV-infected patients are women. Second, all topical DNCB applications were performed by a single investigator, thereby ensuring a standardized treatment methodology for all subjects. Third, the DNCB protocol used in the study differed from the so-called Epstein protocol described previously [7] in that a monthly 10% DNCB booster dose was used to supplement the weekly 2% DNCB applications. This monthly booster may have enhanced the effect of chronic DNCB therapy. Fourth, confounding antiretroviral chemotherapy was not available to the study subjects. Thus, the present trial represents the broadest, purest and most aggressive DNCB evaluation reported to date. Although our study does not conform to the 'intent-to-treat' model used in larger clinical trials, the study results have validity based on the 'pragmatic' design used in smaller trials [17]. The results require confirmation in a larger group of therapy-naive HIV patients.

The incidence of HIV disease in Brazil is among the highest in the world [9–13]. The clinical spectrum of opportunistic infections in Brazilian HIV patients is similar to that of Africa and Haiti, with high rates of tropical, parasitic and venereal diseases [2, 9]. In addition, the rate of HIV disease progression in Brazil is more rapid than in Europe and North America [9]. In spite of its vast natural resources, Brazil has many of the social, economic and medical limitations of poorer countries coping with HIV disease. In particular, therapeutic options for indigent HIV patients are extremely limited due to the high cost of medications and medical care [9, 10]. For example, a course of combination antiretroviral therapy costs a minimum of US$ 830.00 per month. This price is out of reach for most Brazilian HIV patients. In contrast, topical DNCB therapy costs about US$ 1.20 per month. Although even this amount may be excessive for indigent patients, the cost is more feasible than that of standard antiretroviral drugs.

Another advantage of topical DNCB therapy is its ease of use. The weekly application schedule is based on what is known concerning the effect of DNCB on the cellular immune response [18–20]. Topical DNCB induces a delayed-type hypersensitivity skin reaction that in turn stimulates a systemic cellular immune response involving antigen-presenting cells, cytotoxic CD8 T cells and natural killer cells [5, 6]. This cellular immune response has been shown to control HIV replication and disease progression [21–23]. The present study did not evaluate cytotoxic CD8 T-cell subsets, natural killer cells or viral load in DNCB-treated patients. A larger controlled trial of DNCB will examine these immunologic parameters in HIV patients.

In summary, we have performed a standardized, controlled trial of topical DNCB therapy in HIV patients from Brazil. DNCB therapy is associated with improved clinical and immunologic parameters in these patients. The cost of topical DNCB therapy is minimal compared to antiretroviral chemotherapy. DNCB treatment represents a logical and accessible therapeutic approach for developing nations with limited resources to combat the AIDS epidemic.

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Abstract

Treatment of HIV disease in Brazil with dinitrochlorobenzene (DNCB)

Background: This Abstract consolidates the results of a controlled clinical trial using dinitrochlorobenzene (DNCB) to treat HIV disease, which was reported at the 11th World AIDS Conference [Dermatology, 195 (1997) 369-373], with data from two years of additional treatments with DNCB.

Objective: To continue evaluation of the efficacy of topical DNCB as a treatment for HIV disease.

Method: The clinical trial was performed in a public hospital where patients are routinely seen free of charge. Patients of both genders participated. Inclusion criteria were: (1) HIV seropositivity. (2) CD4 T cell counts between 100 and 600 cells/ul. (3) No previous use of DNCB. (4) No current use of antiretroviral therapy. Treatment was initiated with a 2% solution of DNCB in acetone. If there was no sensitization, a 10% solution was used, repeated weekly until there was sensitization. After that, a 2% or 1% solution was applied weekly. Applications were to a 3cmX3cm area on the forearm. A total of 61 patients participated, 35 men and 26 women. The treatment period was two to five years. There were clinical evaluations every three weeks. Peripheral blood lymphocyte subsets were analyzed every six weeks, including CD4 and CD8 cell counts. Viral load was measured only during the last 18 months. DNCB costs less than 1% of antiretroviral therapy.

Results: (1) No patient developed Kaposi sarcoma or any other malignancy. (2) 93% of the patients gained weight. (3) CD4 counts remained stable or increased slightly. (4) CD8 counts increased significantly. (5) Viral load decreased in 82% of the patients. (6) There were few opportunistic infections. (7) There were no side effects other than mild eczema.

Conclusion: The results of this controlled trial of DNCB, together with its very low cost, its ease of application, and the absence of significant side effects, suggest that DNCB deserves much greater emphasis as a treatment for HIV disease, especially in developing nations.

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The Journal of Investigative Dermatology, 110(4) (1998), 476 (Abstr. 21)

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DNCB TREATMENT OF HIV-INFECTED PATIENTS LEADS TO BENEFICIAL IMMUNOLOGIC OUTCOMES, REDUCED VIRAL LOAD, AND IMPROVED MEASURES OF QUALITY-OF-LIFE.

Renato Orasion, Melanie Adams, Krista Sharp, Roger Wilson, Irene Dougherty, Karen Hount, William Epstein and Ponciano Cruz, Jr.
Departments of Dermatology, University of Texas Southwestern Medical Center, Dallas, TX and University of California San Francisco, CA, USA.

Antiretroviral regimens including protease inhibitors can markedly reduce viral burdens in HIV+ patients, but have less success in improving immune status. Contact hypersensitivity provides a theoretical model for enhancing the cellular arm of the immune system, and anecdotal reports and small studies have suggested that chronic application of the contact allergen DNCB can retard progression to AIDS. To evaluate the efficacy and safety of this treatment, we compared effects of weekly skin patch application of DNCB to those of an irritant (SLS) and the vehicle (acetone) in previously unsensitized HIV+ patients on treatment with protease inhibitors for at least 2 mos prior to enrollment. Ninety patients were randomized to the 3 skin patch treatments and evaluated in a double-blinded fashion at 0, 4, 8 and 12 mos. Mean changes in outcomes over time were analyzed using repeated measures of variance and Cox proportional hazards regression. DNCB-treated patients showed steep increments in peripheral CD4 and CD8 T-cell counts (180 and 500 cells/μL respectively) and in scores of skin reactivity to 3 recall Ag. This immune enhancement was paralleled by significant reduction of plasma HIV load and by improvement in 3 of 10 quality-of-life measures (overall health, skin health, and ability to care for self). By contrast, SLS- or acetone-treated patients showed either no change or deterioration in these parameters. There were no adverse effects other than local skin irritation from the patches. Our findings represent the first controlled, randomized and double-blinded validation of the beneficial effects of weekly DNCB application in HIV+ patients. Topical DNCB is a safe and valuable addition to the treatment of these patients.

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Rev Chil Obstet Ginecol 1991;56(6):460-3

[Immunotherapy with cutaneous dinitrochlorobenzene in papillomavirus infection of the cervix uteri].

[Article in Spanish]

Salvo A, Rojas I, Figueroa L, Stuardo P

Servicio de Dermatovenereologia, Hospital Sotero del Rio.

The authors report their experience after the treatment of 30 patients with condyloma acuminatum of endocervix who were diagnosed by cytology (Papanicolaou), colposcopy and biopsy. The women were treated with dinitrochlorobenzene applied on the arm's skin. The sensitization procedure with DNCB, with a cutaneous administration every fifteen days, is described. Some aspects of the HPV infection, the increased number of patients suffering this viral infection and the concern about the relationship between cervical cancer and types 16 and 18 HPV are described. Basic concepts in regard of immunological treatment are discussed. The results were = eighty seven (87%) per cent of the 30 women were fully healed.

PMID: 1669555, UI: 94105472

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

7-1

IND 49,949 Date FEB 15 1996

L. Bruce Mills, M.D.
The Honolulu Medical Group
550 South Beretania Street
Honolulu, Hawaii 96813

Dear Sir or Madam:

We acknowledge receipt of your Investigational New Drug Application (IND) submitted pursuant to Section 505(i) of the Federal Food, Drug, and Cosmetic Act. Please note the following identifying data.

IND Number Assigned: 49,949

Sponsor: L. Bruce Mills, M.D.

Name of Drug: 1- Chloro-2,4-dinitrobenzene (DNCB)

Date of Submission: February 6, 1996

Date of Receipt: February 9, 1996

Studies in humans may not be initiated until 30 days after the date of receipt shown above. If, within the 30-day waiting period, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies until correction, we will notify you immediately that the study may not be initiated ("clinical hold") or that certain restrictions must be placed on it. In the event of such notification, you must continue to withhold, or to restrict, such studies until you have submitted material to correct the deficiencies, and we have notified you that the material you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND. However, if drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

Post-it® Fax Note 7671	Date 4.3.96	# of pages ▶ 2
To Dr. Strickler	From L. Bruce Mills	
Co./Dept.	Co. Honolulu Med. Grp	
Phone #	Phone # 537-2211	
Fax # 415-399-1067	Fax # 521-7080	

You are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the regulations implementing that Act (Title 21 of the Code of Federal Regulations). Those responsibilities include reporting any adverse experience associated with use of the drug that is both serious and unexpected to the FDA as soon as possible and in no event later than 10 working days after initial receipt of the information and reporting any unexpected fatal or life threatening experience to the FDA by telephone no later than 3 working days after receipt of the information (21 CFR 312.32), and submission of annual progress reports (21 CFR 312.33).

Please forward all future communications concerning this IND in triplicate, identified by the above IND number, and addressed as follows:

Food and Drug Administration
Center for Drug Evaluation and Research (HFD-530)
Attention: Document Control Room
5600 Fishers Lane
Rockville, Maryland 20857

Should you have any questions concerning this IND, please contact Vikki S. Kinsey (301) 827-2335

Sincerely yours,

Vikki S. Kinsey

Consumer Safety Officer
Division of Anti-Viral Drug Products
Office of Drug Evaluation
Center for Drug Evaluation and Research

cc: Original IND - pink
HFD-530 - yellow
HFD-530/CSO - green

IND ACKNOWLEDGEMENT

7-3

THE QUEEN'S MEDICAL CENTER

1301 Punchbowl Street • Honolulu, Hawaii 96813 • Phone (808) 538-9011 • FAX: (808) 547-4646

September 19, 1995

L. Bruce Mills, M.D.
The Honolulu Medical Group
550 South Beretania Street
Honolulu; Hawaii 96813

Dear Doctor Mills:

I am pleased to inform you that at its meeting on September 13, 1995, the Research and Institutional Review Committee approved your protocol entitled **"Development of a Topical Delivery System for Dinitrobenzene (DNCB) in HIV Infection"**, contingent upon the Food and Drug Administration issuing an IND for the DNCB Patch.

This approval is in effect for one year from the date of the IRC meeting. Applications for renewal must be made two months prior to the expiration date.

This recommendation has been forwarded to the Medical Executive Committee and the Board of Trustees for their approval. Please be advised that no trials may begin until final approval of the Board has been received.

There are some revisions necessary to the consent form and I attach a copy showing the changes requested. Please may we have an amended copy for our files.

The R&IRC, in conformance with federal regulations, would remind you of the following:

- all correspondence relating to this investigation must be retained;
- any significant changes in protocol must be brought to the attention of the IRC prior to the change being instituted, except in those instances in which patient safety is involved;
- any unexpected adverse reaction must be reported to the IRC and the relevant sponsoring agency; and

Cont'd....p.2

- reports on the conduct of the study are required at least annually with particular reference to adverse reactions, if any.

Yours sincerely,

Carl W. Boyer, Jr., M.D.
Chairman, Research & Institutional
Review Committee

cc: Sally Myers, Dr.PH

CWB:TM:jh

8-1

Cellular Immunity Foundation

(A Nonprofit Public Benefit Corporation)
1370 Taylor Street, Suite 10, San Francisco, California, 94108-1031
Tel: 415-885-6271 Fax: 415-929-7347 e-mail: cifsf@ aol.com

Treating HIV Disease with Dinitrochlorobenzene (DNCB)

Richard De Lancie and Raphael Stricker, M.D.

September 13, 1999

1. DNCB Background.

Studies of DNCB have been performed on humans by many investigators. Starting in the 1920s, DNCB was recognized as a compound that produced skin reactions in factory workers [1,2]. In the 1930s and 1940s, Chase and Landsteiner showed that these skin reactions were caused by white blood cell activation [3] and Sulzberger et al. tried using DNCB to treat various skin diseases [4].

In the 1950s, Epstein and Kligman used DNCB skin testing to evaluate immune function in humans, demonstrating the relationship between skin activity and cell mediated immunity (CMI) [5]. Subsequently, a landmark paper by Catalano et al [6] in 1972 established a simple procedure for immunologic evaluation using DNCB skin testing. This opened the door for DNCB testing in many immunodeficiency diseases over the next 10-15 years and during this time DNCB was explored as a treatment for skin diseases that might be subject to immune modulation, such as warts, baldness and skin cancers [7]. These studies laid the foundation for utilizing DNCB to boost immune function in HIV disease.

Starting in the 1980s there have been a series of pilot studies and clinical trials of DNCB as a treatment for HIV disease [8]. These are described in section 6.

2. Conventional treatments of HIV disease.

The conventional treatment for HIV disease in the industrialized countries is antiretroviral therapy (ART), using combinations of nucleoside analogs, non-nucleoside analogs, and protease inhibitors. Recently, a particular version of ART, Highly Active Antiretroviral Therapy (HAART) has become the standard of care for HIV disease [28]. HAART requires that high doses of antiretrovirals, including a protease inhibitor, be taken in accordance with a rigorous medication schedule starting as soon as HIV infection is detected.

The introduction HAART has resulted in a dramatic improvement in the longevity and quality of life for hundreds of thousands of HIV patients, almost all who live in the industrialized world and are in a position to receive those treatments. But as is well established in the literature, those treatments come with severe limitations:

- Some patients receive no benefits from HAART.
- Some patients have such severe side effects that they can not continue HAART.

- Many patients find the medication schedule so demanding that they do not comply with it, with the usual result that HIV mutates to resistant forms not affected by HAART.

- The result is that, all told, it is estimated that about one third of those who have access to HAART do not or can not use HAART.

- The cost of the drugs alone for HAART is on the order of $15,000 per year.

- For over 95% of those in the World infected with HIV, neither they nor their governments can afford HAART.

3. Another, more logical, approach to HIV disease

A more logical approach to HIV disease is based on the idea that it is not HIV, the virus, that's the problem, it's the "body's response to the virus". The implication of this phrase is that it the human immune system, and cellular immunity in particluar, is the key to fighting HIV disease. Antiretroviral drugs can't boost the cellular immune response for any appreciable time. Treatments are needed that goad the human immune system into action against HIV, especially in such places as the skin and the lymph nodes where the virus likes to hide.

This can be done with an immune booster. The most widely used immune booster is a simple chemical compound called dinitrochlorobenzene, or DNCB. DNCB has been used in the development of color photography for the better part of a century. When applied to the skin, DNCB causes a type of allergic reaction known as delayed –type hypersensitivity (DTH). It is called "delayed-type" because there is a typically a delay of two to three days between the time the skin is exposed to the allergen (DNCB in this case) and the appearance on the skin of a reaction to the allergen. An example of an allergen and the skin reaction to it is seen in the case of poison oak or poison ivy.

This localized skin reaction is accompanied by a response throughout the immune system, particularly in the lymph nodes that stimulates the cells that fight HIV. Applications of DNCB on a weekly basis creates a persistent cellular immune activation that controls HIV disease. Best of all, the cost of the DNCB is about a dollar a month, largely because it is very cheap to make and is in the public domain.

Why, then, isn't DNCB used by the medical establishment around the world?. For one thing, DNCB is so cheap that no pharmaceutical company could profit from its use even remotely to the extent that such companies profit from the sale of antiretroviral drugs, which are proprietary products. That has grossly hindered the use of DNCB as commercially available treatment for HIV disease.

Another problem is that DNCB does not directly kill HIV. Instead, it works by boosting the human cellular immune system. That concept is not easily accepted by virologists and pharmaceutical companies that are devoting tremendous efforts to finding drugs that will directly or indirectly kill, or prevent the replication, of HIV.

A third problem (or advantage, depending on the patient's orientation) is that DNCB is not a pill or an injection. It is in the form of a solution that has to be rubbed on the skin. This approach to treatment, although common in lesser-developed societies, does not conform to the standard guidelines of Western medicine. That is an advantage worldwide, however, since well over 90%

of those infected with HIV worldwide live in lesser-developed societies, where the DNCB mode of treatment should be more acceptable than in the West.

In short, DNCB represents a logical approach to the treatment of HIV infection. It is certainly not a cure for HIV disease. In fact, as with antiretroviral therapy, with or without IL2 regimes, DNCB treatment must continue for a lifetime. But it is a lifetime of simple weekly skin treatments rather than the daunting medication schedule involved with antiretroviral therapy. Furthermore, it is so cheap that it can be made available to every infected person in the world, not just the very minor fraction of the HIV infected that live in industrialized countries.

4. How DNCB works in treating HIV disease.

Several possibilities regarding the immunological profile of DNCB in human beings have been proposed in the past. One explanation was based on the knowledge that the Langerhans cells of the epidermis and dendritic cells throughout the body are infected with HIV and that the infected cells have an impaired antigen-presenting capacity [21-23]. Exposure to DNCB was thought to correct the dysregulation that develops between the infected antigen-presenting cells and T-cells, leading to control of HIV replication in various organ systems [8].

Another explanation considered was that repeated exposure to DNCB amplifies the Th1 CD8 + effector T-cell subset that enhances cell-mediated immunity (CMI). Enhanced CMI promotes the type of cell function and cytokine production that may be necessary for survival for a person infected with HIV [8, 24-27]. It was speculated that upregulartion of the Th1 response by DNCB could reverse the pathological 'Th1/Th2 switch' described in HIV patients [26], with consequent mobilization of cytotoxic CD8+ T cells and natural killer (NK) cells. Two other very important studies have shown that survival is very dependent on a higher level of CD+ T cells and very little dependent on the CD4+ level [29,30], particularly important because DNCB therapy typically results in a substantial increase in the CD8+ level. Other perspectives regarding the contact sensitization profile of DNCB appear in the protocols of the clinical trials being conducted by Dr. Mills [16] and Dr. Cruz [17].

Very recently, a more detailed understanding of how DNCB works is emerging. The understanding follows from studies of the signaling molecules known as cytokines and chemokines that are produced by the immune system in response to HIV infection. These studies show how the known effects of DNCB result in the initiation of a cascade of cellular immune modulators that limit the effects of HIV disease, with the result that uninfected cells kill or neutralize HIV infected cells. The cascade continues as long as DNCB is used regularly, maintaining an ongoing attack against HIV.

5. When protocols are followed, DNCB results in no serious side effects.

Like coffee, tea, and celery, DNCB causes chromosome mutations in bacteria [18]. However, studies in laboratory animals and humans have conclusively shown that DNCB is <u>not</u> a carcinogen [19] or a mutagen in mammals. In fact, during the 70 years that DNCB has been used for medical purposes in humans, there is no evidence that cancers have developed as a result of applying DNCB to the skin. In AIDS patients, excessive applications of DNCB have resulted in toxic skin reactions [20].

For some time, however, DNCB dosage has been standardized, using the Epstein protocol. When this protocol is used, excessive skin reactions have been effectively controlled with local measurers, such as calamine lotion and cold camomile compresses. [12-14]. No infections have

been seen as a result of these reactions. Other indications of the tolerance of humans to treatment with DNCB can be found in the foregoing descriptions of pilot studies and clinical trials of DNCB therapy [8,12-14].

6. Therapeutic effectiveness of DNCB for the treatment of HIV disease has been demonstrated in pilot studies and controlled clinical trials.

a. Pilot Studies

In the first pilot study of DNCB, involving 16 patients [9], the results were the following:

- DNCB application on a weekly basis resulted in increased numbers of cytotoxic CD8 +effector T-cells and natural killer cells in-patients at various stages of HIV disease. (Other studies had shown that elevated CD8 effector T-cells and natural killer cells [8,10,11] are important in prognosticating survival in HIV disease.)
- No patients had progression of their disease.
- Two patients had excessive skin reactions to DNCB. Those reactions were easily treated with topical creams.

The following was observed in a study of 24 patients with early-stage HIV disease sensitized with DNCB [12].

- Treatment with DNCB elevated cytotoxic CD8 + effector and natural killer cell level-s.
- There were significant decreases in viral load for patients treated with DNCB whose viral load before treatment was greater than 10,000 copies per ml. There were no changes in viral load for patients whose viral load before treatment was less than 10,000 copies per ml.
- Toxic skin reactions to DNCB were not seen.

There was a follow-up study [8] of the 24 patients in the study described above (all of whom had been treated with DNCB). Eleven had discontinued treatment after nearly a year. The remainder had continued DNCB treatment until the end of the study, over a year later. The results were as follows:

- There were significant increases in CD8 + effector and natural killer cells in patients who continued treatment and significant decreases in those who had discontinued treatment.
- There were superior clinical outcomes for the 13 patients who continued treatment (two developed AIDS and none died), as compared with the 11 patients who discontinued treatment (five developed AIDS and four died).

In addition to these pilot studies, it is important to note that for well over ten years thousands of patients with HIV disease have been using DNCB on a self-prescribed basis [8]. Physicians in contact with many of those users, including those with extensive HIV/AIDS experience, report the following:

- Survival rates, rates for opportunistic diseases, and general health appear to have been considerably better than for the untreated. (Many say that they have also been better than for those treated with antiretroviral therapy.)

- There are no reports of adverse effects with use of the standard dosage.

- In summary, DNCB applications have been well-tolerated [8]

There has been a pilot study of the effect of DNCB in monkeys at the Karolinska Mediko-Kirurgiska Institut in Stockholm. The monkeys were infected with simian immunodeficiency virus (SIV) [in press]. A group of nine cynomolgus macaque monkeys was infected with SIV. Five were treated and four were not. The results were as follows:

- All monkeys appeared healthy for 12 months.

- By 16 months, all the monkeys treated with DNCB remained healthy, but three of the four untreated monkeys died of lymphoma.

b. Controlled Clinical Trials

The following results were reported from a controlled clinical trial in Brazil [14], without any significant adverse effects being noted:

- Patients treated with DNCB gained significant weight while untreated patients lost significant weight.

- Treated patients had significantly higher CD4 and CD8 + effector T cell counts than did control patients.

- Only 4% of treated patients progressed to AIDS, while 40% of control patients progressed.

- All of the untreated patients had infectious complications during the trial. Only 21% of the control patients had those complications.

A three-year extension of the foregoing trial was reported on at the 12th World AIDS Conference in Geneva in June 1998. The following results were reported, involving 61 patients, some over two years and others over five years:

- No patient developed Kaposi sarcoma or any other malignancy.

- 93% of the patients gained weight.

- CD4 counts remained stable or increased slightly.

- CD8 counts increased significantly.

- Viral load decreased in 82% of the patients.

- There were few opportunistic infections.

- There were no side effects other than mild eczema.

Another clinical trial with 90 patients is underway

The effect of DNCB on viral load has also been the subject of a controlled clinical trial [15]. Eight patients were treated with DNCB and six were untreated. The trial lasted three to four months. The results were as follows:

- During the short period of the trial, the mean CD4 and CD8 + effector T-cell levels increased in the treated group and decreased in the untreated group, but the changes were not statistically significant.
- There was a significant decrease in the mean plasma viral load of the treated group and a significant increase in the mean viral load of the untreated group.

7. Additional NIH or FDA controlled clinical trials are underway.

Controlled clinical trials of DNCB are underway at two locations in the U.S. One is in Honolulu, Hawaii, where L. Bruce Mills, M.D., of the University of Hawaii, is the Principal Investigator [16]. It was Dr. Mills who, over ten years ago, did the first pilot study on DNCB, when he was a Research Fellow at Stanford University. Dr. Mills's trial is being carried out under an "Investigational New Drug Application (IND)" of the U.S. Food and Drug Administration (FDA). The purpose of the clinical trial in Honolulu is to evaluate the efficacy of delivering DNCB by a skin patch, rather than by applying it to the skin in an acetone solution.

The first phase of a second clinical trial of DNCB has been completed at the University of Texas Southwestern Medical Center of the University of Texas at Dallas [17]. The Principal investigator there is Ponciano D. Cruz, Jr., M.D. That trial has been funded by a grant from the U.S. National Institutes of Health (NIH). The clinical trial in Dallas resulted from the belief that inexpensive dermatologic intervention with DNCB may have a profoundly positive influence on morbidity and mortality in HIV disease.

The clinical trial in Dallas was designed to analyze the combined effects of DNCB and antiretroviral therapy on patients with HIV disease and to investigate the relationships among HIV infection, skin diseases, and therapeutic interventions. In a randomized double blind study of 90 patients the effects of weekly skin patch applications of DNCB were compared with those of an irritant (SLS) and of the vehicle (acetone). The following results of the first phase were presented at a meeting in May:

- DNCB-treated patients showed steep increments in peripheral CD4 and CD8 T-cell counts and in the scores of skin reactivity to 3 recall Ag.
- That immune enhancement was paralleled by significant reduction in of plasma HIV load and by improvements in patients overall health, skin health, and ability to care for self.
- SLS- or acetone-treated patients showed either no change or deterioration in their parameters.
- Preliminary indications are that there have been very significant benefits from the addition of treatment with DNCB to patients already being treated with antiretroviral therapy.

7. Summary:

- DNCB has been in use for a variety of medical purposes for 70 years.

- Due to that extensive usage, it is extremely unlikely that any adverse effects, other than due to overdosing, will come to light.

- When the established protocol is used, overdoses occur only occasionally, are easily treated, and do not lead to infections.

- The theoretical basis for the effectiveness of DNCB is now understood.

- Controlled clinical trials of DNCB have demonstrated very clearly both comparative clinical effectiveness and absence of significant adverse effects.

- Controlled clinical trials were preceded by a number of pilot studies that also demonstrated effectiveness and absence of significant adverse effects.

- Controlled trials have had as Principal Investigators clinicians with extensive experience in treating HIV disease.

- Thousands of HIV infected patients in the U.S. and elsewhere have been treating themselves with DNCB for many years and there is extensive evidence to support the effectiveness and safety of those treatments.

- There are currently only three alternatives to treatment with DNCB: (1) no treatment (2) treatment with HAART, and (3) preventive vaccines.
  - With no treatment, HIV disease leads in most cases to AIDS and then to death.
  - Treatment with HAART leads to many severe problems with antiretroviral drugs [43], including serious side effects, ineffectiveness due to HIV mutations, multidrug resistance, very demanding medication schedules, and very high cost. (It is estimated that at least one third of the infected can not take or do not benefit from antiretroviral drugs for reasons other than their high cost.)
  - The prospect for a preventive vaccine in the next ten years is not encouraging [44].

- Even if HAART were completely effective, the very high cost of HAART prevents over 95% of those in the World infected with HIV from benefiting from it.

- For those who can afford and tolerate HAART, DNCB appears to enhance HAART'S efficacy.

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Pilot study of topical dinitrochlorobenzene (DNCB) in human immunodeficiency virus infection

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1. Summary

Dendritic cells, the primary antigen presenting cells of the human immune system, are heavily infected with human immunodeficiency virus (HIV) in patients with the acquired immunodeficiency syndrome (AIDS). Dinitrochlorobenzene (DNCB) is a contact sensitizing agent that acts as a potent immune modulator of dendritic cells. In this pilot study, we examined the safety and efficacy of topical DNCB application in patients with early HIV disease. Topical DNCB was well tolerated by these patients, with an adverse reaction rate of 10%. $CD4^+$ T-cell counts remained stable with repeated DNCB use. In contrast, $CD8^+$ T-cell counts and natural killer cells increased significantly following DNCB sensitization. This increase in $CD8^+$ T-cell and natural killer cell subsets was accompanied by a decrease in HIV replication, as measured by serum HIV RNA levels. Based on this pilot study, we conclude that topical DNCB is safe in early HIV disease and may decrease viral load via a systemic effect on dendritic cells, $CD8^+$ T-cells and natural killer cells. These results require confirmation in larger controlled trials.

Key words: Dinitrochlorobenzene; HIV; AIDS; Dendritic cell; Langerhans cell; Antigen presenting cell

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2. Introduction

Dendritic cells and epidermal Langerhans cells are blood and tissue mononuclear cells that function as the primary antigen presenting cells of the human immune system [1–5]. Depletion and dysfunction of these cells have been demonstrated in early stages of the acquired immunodeficiency syndrome (AIDS) [6–10]. Recently, dendritic cells have been shown to be heavily infected (up to 25%) with human immunodeficiency virus (HIV) in patients with AIDS [9,10]. Thus, dendritic cells serve as a major reservoir for HIV, and dendritic cell abnormalities contribute significantly to the immune dysfunction seen in AIDS.

1-Chloro-2,4-dinitrobenzene (DNCB) is an organic compound that is used in color photography. Like other contact sensitizing agents, DNCB is a potent modulator of dendritic cell function in vitro, and it has been used to stimulate cellular immune function in vivo [1,11,12]. Because topical application of this compound has a systemic effect on the cells that form the major reservoir for HIV [1], we organized a pilot study to examine the safety and immunologic efficacy of topical DNCB use in HIV infection.

3. Study design

3.1. Study subjects

Subjects were enrolled in the study after informed consent was obtained. Inclusion criteria were as follows: HIV seropositivity with $CD4^+$ T-cell counts between 100–600 cells/μl; no prior use of DNCB; either no current use of antiretroviral therapy, or treatment with a stable dose of zidovudine (AZT), dideoxyinosine (ddI) or dideoxycytidine (ddC) for at least six months; and no use of any other experimental medication. Patients with an AIDS diagnosis (significant opportunistic infections or malignancy) were excluded. All subjects had routine follow-up with their primary physicians during the study, and blood test results were shared with the primary physician.

3.2. DNCB protocol

The protocol was designed as an observational study of topical DNCB application by the study subjects. DNCB was supplied in an acetone solution in concentrations of 10%, 2%, 0.2% and 0.02% (Healing Alternatives Foundation, San Francisco, CA). Once subjects were enrolled in the study, baseline blood samples were drawn for lymphocyte subset testing, and serum was banked at -70°C. Each subject then underwent patch testing with a 2% DNCB solution applied to a 5 mm skin site. The patch test was used to rule out prior sensitization to DNCB. If the patch test was negative, each subject then proceeded to the sensitization step by applying the 10% DNCB solution to a 2.5 cm area of skin, usually on the forearm. Reactivity was achieved when contact dermatitis occurred at the site within 72 h of DNCB application. If reactivity did not occur with the original 10% solution, subjects repeated this dose at weekly intervals until sensitization occurred.

Once reactivity was demonstrated, subjects began weekly application of 2% DNCB to a 2.5 cm site located on the forearms or upper arms. With repeated application, a 'reflare' (reddening or itching at the original application site) was seen. This response confirmed systemic sensitization to the DNCB. Subjects then decreased the weekly dose to the lowest amount that elicited a localized contact dermatitis.

Subjects kept a log of all symptoms during DNCB use. Clinical evaluation was performed every two months by the protocol coordinator.

3.3. Lymphocyte subset analysis

Peripheral blood lymphocyte subsets were analyzed using monoclonal antibodies (Coulter Corporation, Hialeah, FL) and two-color flow cytometry, as previously described [13]. The subsets that were analyzed included helper/inducer T-cells ($CD4^+$), suppressor/cytotoxic T-cells ($CD8^+$), cytotoxic T-cells ($CD8^+CD57^+$) and natural killer cells ($CD56^+$). Blood samples were drawn every two months during the study, and lymphocyte subset analysis was performed within 24 h of blood drawing.

3.4. Serum HIV RNA quantitation

Quantitation of HIV RNA in banked serum samples was performed using reverse transcriptase (RT) and the polymerase chain reaction (PCR), as previously described [14,15]. In brief, total RNA was extracted from serum using RNazol (Biotecx Laboratories, Houston, TX). HIV RNA was reverse transcribed using random hexamers, amplified by PCR, and then detected by oligonucleotide hybridization [15]. Using a standard curve of synthetic HIV RNA, the amount of HIV RNA in serum samples could be quantitated over a linear range from 50 to 1000 input copies.

Serum samples were analyzed for HIV RNA prior to DNCB sensitization and then two months after sensitization. In addition, HIV RNA quantitation was performed in select cases every two months over the course of the study.

3.5. Statistical analysis

The data was not normally distributed. Therefore, the results were analyzed by nonparametric methods using the Wilcoxon Signed Rank Test.

4. Results

The clinical characteristics of the study subjects

TABLE 1

Clinical characteristics of study subjects.

Total:	20
Asymptomatic:	16
Lymphadenopathy:	4
Medications:	
None:	11
AZT:	9
AZT + ddI	1
AZT + ddC	1
Mean $CD4^+$ T-cell counts:	353/μl
Range:	170–560/μl

are shown in Table 1. Twenty homosexual men were enrolled in the study. Sixteen were asymptomatic, while four had lymphadenopathy. Nine patients were on a stable dose of AZT, while eleven patients were on no medication. One subject was on a stable dose of ddI and one was on a stable dose of ddC in addition to AZT. $CD4^+$ T-cell counts ranged from 170/μl to 560/μl prior to entry into the study.

All patients underwent DNCB sensitization, and all were sensitized by the 10% solution. Two subjects experienced significant reactions to the initial DNCB application despite negative patch tests. These reactions included burning, itching and erythema at the application site (Fig. 1). The reactions subsided within 48 h with local care (moisturizing lotion or cream). No subject had a systemic adverse reaction to the DNCB, although most subjects experienced some initial discomfort at the application site. Each subject experienced a 'reflare' within 3–6 weeks after initiation of the 2% DNCB applications.

Sixteen subjects were followed for 3–20 months after DNCB sensitization. The mean follow-up was seven months. None of the subjects had progression of HIV disease during this time. One patient withdrew from the study because of the 'cosmetic effects' of DNCB application. Another patient discontinued DNCB use because of discouragement by his private physician. These subjects were followed for six months after discontinuing DNCB. Neither subject was on AZT.

Results of lymphocyte subset analysis in the 16 patients are shown in Table 2. The mean $CD4^+$ T-cell count prior to DNCB use was 347 cells/μl. Following DNCB sensitization, the mean $CD4^+$ T-cell count remained stable at 343 cells/μl (P = N.S.). Subjects on AZT had a slight increase in $CD4^+$ T-cells, while subjects not on AZT had a slight decrease. These differences were not significant, however. In contrast to the $CD4^+$ T-cell counts, $CD8^+$ T-cell counts showed a significant increase following DNCB sensitization, from a mean of 904 cells/μl to a mean of 1068 cells/μl (P

TABLE 2

Effect of topical DNCB application on lymphocyte subsets.
N, number of patients; N.S., not significant. Normal ranges: $CD4^+$ T-cells, 400–1800/μl; $CD8^+$ T-cells, 300–1200/μl; $CD8^+CD57^+$ T-cells, 80–200/μl; $CD56^+$ natural killer cells, 150–400/μl. SEM, standard error of the mean.

Lymphocyte Subsets (Cells/μl)	N	Pre-DNCB (Mean ± SEM)	Post-DNCB (Mean ± SEM)
$CD4^+$ (Helper/Inducer)	16	347 ± 30[a]	343 ± 31[a]
-On AZT	7	260 ± 31[a]	273 ± 43[a]
-No AZT	9	414 ± 32[a]	397 ± 35[a]
$CD8^+$ (Suppressor/Cytotoxic)	16	904 ± 112[b]	1068 ± 106[b]
-On AZT	7	643 ± 148[c]	836 ± 140[c]
-No AZT	9	1107 ± 133[d]	1249 ± 130[d]
$CD8^+CD57^+$ (Cytotoxic)	16	229 ± 41[a]	244 ± 37[a]
$CD56^+$ (Natural Killer)	16	95 ± 12[e]	119 ± 15[e]

[a]P = N.S.; [b]P = 0.006; [c]P = 0.031; [d]P = 0.046; [e]P = 0.037.

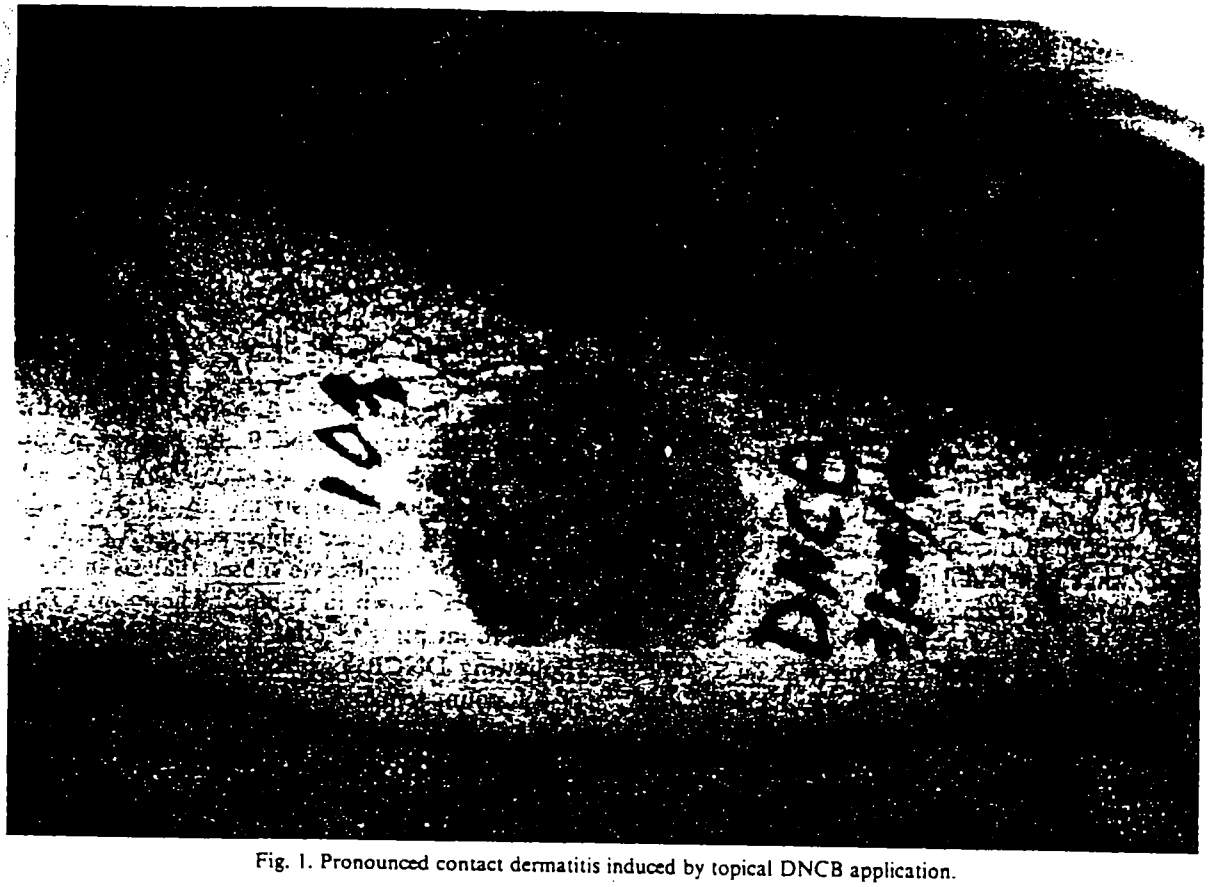

Fig. 1. Pronounced contact dermatitis induced by topical DNCB application.

= 0.006). This increase was seen in patients regardless of AZT treatment (Table 2). The increase in $CD8^-$ T-cells persisted for at least six months during continuous DNCB use (data not shown). Analysis of the cytotoxic T-cell subset ($CD8^-CD57^-$) showed no change with DNCB sensitization (Table 2). In contrast, the natural killer cell subset ($CD56^+$) increased significantly with DNCB use, from a mean of 95 cells/μl to a mean of 119 cells/μl ($P = 0.037$). This increase was independent of AZT treatment (data not shown).

Quantitation of serum HIV RNA in 11 subjects during DNCB use is shown in Table 3. In the two patients who discontinued DNCB application, the mean HIV RNA level increased from 2650 copies/ml to 8800 copies/ml. In contrast, the nine patients who continued to use DNCB showed a significant decrease in HIV RNA levels, from 42911 copies/ml to 16111 copies/ml ($P = 0.02$). Patients on AZT had a slight increase in HIV RNA copies, while patients not taking AZT showed a threefold decrease in HIV RA copy numbers ($P = 0.017$). Subjects with initial HIV RNA copy numbers > 10000/ml showed a greater response to DNCB than subjects with initial HIV RNA copy numbers < 10000/ml (Table 3).

5. Discussion

Although HIV infection is associated with progressive cellular immune dysfunction, the mechanism of this dysfunction is poorly understood [16]. $CD4^+$ T-cells are depleted during the course of HIV disease, but less than 0.2% of these cells become infected with HIV [17]. Recently it has been shown that up to 25% of dendritic cells, the primary antigen presenting cells of the immune system, are infected with HIV [8–10]. Infection of these cells leads to dysfunction and loss of antigen

TABLE 3

Effect of topical DNCB application on HIV RNA levels in serum.
N, number of patients; N/A, not applicable due to small sample size. SEM, standard error of the mean.

Patients	N	Serum HIV RNA (Copies/ml)	
		Pre-DNCB (Mean ± SEM)	Post-DNCB (Mean ± SEM)
Discontinued DNCB[a]	2	2650 ± 1750[b]	8800 ± 800[b]
Continued DNCB	9	42911 ± 17041[c]	16111 ± 5424[c]
-On AZT	2	4800 ± 400[b]	5000 ± 3600[b]
-No AZT	7	53000 ± 20221[d]	19286 ± 6500[d]
RNA < 10000	5	4220 ± 894[b]	6840 ± 1444[b]
RNA > 10000	6	61733 ± 22007[e]	21400 ± 7273[e]

[a]Not on AZT; [b]P = N/A; [c]P = 0.02; [d]P = 0.017; [e]P = 0.014.

presentation, which may be the most significant factor in the development of cellular immunodeficiency in AIDS [10]. Thus, a treatment modality that promotes normal antigen presentation in HIV disease is desirable.

DNCB exerts a significant immunomodulatory effect on antigen presenting cells in vitro and in vivo [1,4,11]. The effect of topically applied DNCB appears to be systemic in nature, with cellular immune responses appearing at distant sites following DNCB application [1,12,18]. Dendritic cells exposed to DNCB in vivo and in vitro stimulate $CD8^-$ T-cells and natural killer cells [19,20]. Expansion of these lymphocyte populations in response to DNCB may be extremely useful in vivo, since HIV suppressor cells may be activated as part of this response, and viral replication may thus be controlled [21,22]. A concern about DNCB use is that $CD4^+$ T-cells would also be activated, leading to increased viral infection of these cells (the so-called 'food for the virus' theory) with a fall in $CD4^-$ T-cell counts and propagation of the disease [23,24].

Our pilot study of topical DNCB use in patients with early-stage HIV disease demonstrates that DNCB is safe in these patients. The local complication rate from application of the compound was 10% (2/20 subjects), and this type of complication was easily treated with local measures. Extensive allergic contact dermatitis has been reported with excessive DNCB application [25], but this problem was not seen when DNCB was used according to the study protocol. Although each patient in our study experienced a 'reflare' (systemic response) with the DNCB regimen that was used, the optimum DNCB treatment dose in individual subjects has not been determined.

A significant finding of our study was that DNCB application did not result in depletion of $CD4^+$ T-cells. This observation appears to invalidate the 'food for the virus' theory of increased $CD4^-$ T-cell destruction due to DNCB. More important, DNCB sensitization was associated with a significant rise in $CD8^-$ T-cells and natural killer cells (Table 2), presumably via dendritic cell modulation. Although the quantitative expansion of these lymphocyte populations persisted over time, the qualitative antiviral properties of these $CD8^+$ T-cell and natural killer subsets were not defined. Further study of HIV suppressor subsets in DNCB-treated patients is now in progress.

DNCB application had a significant effect on viral replication, as shown by serum HIV RNA levels (Table 3). These levels decreased with chronic DNCB use, while they appeared to increase in patients who discontinued DNCB. Patients with higher viral loads (> 10000 copies/ml) had a greater response to DNCB, while patients with lower amounts of circulating HIV RNA (< 10000 copies/ml) maintained these levels over time (Table 3). These results are consistent with the expansion of $CD8^-$ T-cell and natural killer cell subsets that may control viral replication in

response to DNCB.

In summary, topical DNCB application appears to be safe in patients with early HIV disease. Use of DNCB does not cause $CD4^+$ T-cell depletion, and it is associated with an increase in $CD8^-$ T-cells and natural killer cells. This effect is accompanied by a decrease in viral replication, as measured by serum HIV RNA levels. The results of this pilot study require confirmation in larger controlled clinical trials, and the effect of DNCB on HIV suppressor cells merits further evaluation. Testing of this inexpensive, readily available compound in patients with more advanced HIV disease, as well as in seronegative at-risk subjects [26], is also warranted.

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Hit harder than HAART

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Sir--In the introduction to their article on treatment for HIV-1, Mark Harrington and Charles Carpenter (June 17, p 2147)[1] say: ". . . no available regimen can eradicate HIV-1; all currently effective regimens may cause undesirable, sometimes life-threatening, toxic effects; and, unless regimens are strictly adhered to, multidrug resistance can develop, limiting future treatment options." One would conclude that all three of these features had been confirmed beyond question. This is not the case. Treatment is available for which the second and third features do not apply. This treatment is called topical immune modulation (TIM); the modulator is dinitrochlorobenzene. Many encouraging articles about this treatment have been published in peer-reviewed journals.[2-4] These articles include reports on several pilot studies and two clinical trials. All show that TIM works.

The need for treatments such as TIM was emphasised by Joshua Lederberg, when he wrote ". . . our focus on extirpating the virus may have deflected less ambitious, though more pragmatic, aims, including learning to live with the virus by nurturing in equal measure the immune system that HIV erodes.'[5] TIM does just that. Furthermore, with TIM there are no significant side-effects, there is no multi-drug resistance, and difficulties with compliance are minimised. Perhaps more important, dinitrochlorobenzene costs less than 1% of HAART drugs, so TIM can be afforded worldwide, and no technical or complicated medical delivery system is required.

At a time when the world is wringing its hands about a crisis that is threatening both US national security and the capacity of some African countries to function, one would think that anything offering some ray of hope would generate intense interest. For this reason I cannot understand why Harrington and Carpenter do not acknowledge TIM. When I ask people why there is so little interest in TIM, a common response is that TIM has not proved itself as a stand-alone treatment. What they mean is that it has not been subjected to controlled, randomised, double-blind clinical trials, which is true. Funding a trial of a substance that does not have a significant profit potential is extremely difficult. Because dinitrochlorobenzene is in the public domain, it lacks that potential. So pharmaceutical companies show little interest in dinitrochlorobenzene and, as a consequence, funds have not been available for the necessary research and clinical trials to establish it as a standard of care. That, in turn, has meant that TIM, the only treatment now available that could make a difference in Africa, has been ignored, with catastrophic consequences.

Until those who have made HAART the standard of care re-examine their emphasis on HAART, there is unlikely to be a change. If Harrington, Carpenter, and others playing a part in defining treatments for HIV disease take a serious look at TIM, particularly for its potential in the developing world, there may be hope for Africa. None now exists.

Richard De Lancie

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Early report

SURVIVAL, NON-ABORTIVE EFFECTS AND BODY WEIGHT CHANGES IN MICE AFTER EXPOSURE TO DINITROCHLOROBENZENE DURING PREGNANCY.

Paul Urso, Yungri G. Wirsiy, Raphael B. Stricker and Silvio B. Margulis

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Summary

Background. Most therapeutic agents, including 3'-azido-3'-deoxythymidine (AZT), have side effects that limit their use in HIV infected pregnant patients. Dinitrochlorobenzene (DNCB) has been considered as a therapeutic agent because of its up-regulatory activity of cell-mediated immunity (CMI) and its apparent minimal toxic effects. CMI deficiency is a prime feature in mothers and infants infected with HIV. The objective of this work is a preliminary toxicologic evaluation of DNCB therapy in primiparous mice and their progeny when this agent is applied topically during pregnancy.

Methods To evaluate the potential teratogenicity of DNCB we examined survival and body weights of mothers and progeny after topical administration of DNCB at different doses and times throughout pregnancy. DNCB was first applied either at 3 days of pregnancy or at 12 days of pregnancy, and then every other day until parturition. Doses ranged from 2.25 ug/ml to 90 ug/ml in the two groups of mice.

Findings Reactions were observed with each DNCB dose at site of application in the pregnant females. Neither survival nor body weight was affected in the primiparous animals. However, in their

progeny there was a direct relationship between body weight gain and dose of DNCB. Lower doses of DNCB, led to reduction of body weight gain. Survival of progeny was not affected, but litter size from mothers receiving the lower doses was slightly increased. Also, fetuses were not aborted at any of the DNCB doses.

Interpretation Paradoxically it appears that higher doses of DNCB, within the limits of this study, when applied to the mothers during pregnancy, may be less toxic to the progeny during gestation, at parturition, and postnatally. Thus, this CMI stimulator may prove to be an effective therapeutic agent and less toxic, including non-abortive action, at higher dosages in HIV infected patients. Further trials are needed to test the efficacy of DNCB in pregnancy.

Lancet 1996;**XXX:** pg - pg

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Introduction

Human immunodeficiency virus (HIV), the etiologic agent of the acquired immunodeficiency syndrome (AIDS), induces depletion and

dysfunction of antigen presenting cells (APC), such as dendritic cell, epidermal Langerhans cells and macrophages[1]. Since APC abnormalities can lead to defects of cell-mediated immunity and in consequent depletion of CD4 T cells[2], the ability of HIV to thrive may be related to the immunologic disruption of these APC.

1-Chloro-2,4-Dinitrobenzene (DNCB) is a contact sensitizing agent that induces delayed-type hypersensitivity[3], up-regulates APC function[4], and promotes $CD8^+$ cytolytic T cell function[5]. In recent trials with HIV-infected patients, chronic treatment with DNCB has been shown to decrease HIV replication, to increase $CD8^+$ T cell activity and to ameliorate progression of HIV disease, seemingly without undue toxic effects[6,7].

Although reduced maternal-fetal transmission of HIV with 3'-azido-3'-deoxythymidine (AZT) has been reported[8], this agent is severely toxic to the hematopoietic system[9], and also can reduce cell-mediated immunity in the offspring of pregnant mice[10]. Measures to minimize toxicity of AZT have been introduced[11,12], but therapeutic use of this anti-viral drug still has its limitations. Agents with apparently less toxic effects are now in trial phase. In this regard DNCB is currently being considered as a beneficial therapeutic agent in AIDS patients because of its minimal toxic effects[13] and its up-regulation of the cell-mediated immune system.

In terms of supporting anti-viral activity and even preventing transmission of HIV in offspring of pregnant mothers, DNCB may have significant benefits. Although trials with DNCB in AIDS patients are now in progress, as regards maternal-fetal efficiency

for preventing HIV infection of the offspring it is necessary to determine the kinds of toxic side effects this agent may have on the proger . Thus, as a first step, we assayed body weights of the offspring after topical application of DNCB to the skin of pregnant mice. Survival of pups and mothers was also determined.

Methods

In testing DNCB on the body weights of the progeny, 2 approaches were employed. In the first approach, the contact sensitizer was applied to a 1 inch square area of skin behind the ears of pregnant C3HeB/J adult mice (8 weeks of age) on the 12th day of pregnancy (beginning second trimester) after shaving off the hair. DNCB was applied every other day until parturition. In the second approach, DNCB was first applied to the shaved area on the third day of pregnancy (after observation of a vaginal plug considered as day 0, beginning of the first trimester) and every other day thereafter until parturition. The drug was not applied after the pups were born. Three doses of DNCB dissolved in 95% ethanol were used: a) 90 ug/ml; b) 22.5 ug/ml, and c) 2.25 ug/ml as the initial application, and thereafter the same dosages as the initial dose were used for all three groups. These doses roughly correspond to ratios of 4:1, 1:1, and 1:10 compared to the maintenance dose commonly used in human subjects[3]. A control group received ethanol only. The volume applied was 0.5 ml and the ethanol was allowed to evaporate quickly using a fan to the area of application. The mice were not anesthetized while the drug was applied, and no licking of

the area occurred upon observing the mouse for at least 3 hours after application. Statistical evaluation was performed using analysis of variance, and significance was established by Student's t-test using Dunnett's modification for comparison of all 4 groups and Bonferroni's modification where one group was compared to another.

Results

Two females in each group were used for topical application on day 12 of pregnancy, and 1 female per group was used for application on the 3rd day of pregnancy. All the animals demonstrated skin reactions to DNCB. DNCB did not appear to affect the weights of the pregnant mothers to any large degree whether it was applied on the 12th day or on the 3rd day of pregnancy (Table). Generally, litter size and pup survival was not appreciably different from the control group, indicating that DNCB at any dose did not lead to abortions. This was also apparent from no significant change in liiter size of mothers receiving 90 ug DNCB/ml and from the increase in litter sizes of mothers receiving the lower doses. These litter sizes were slightly larger than controls or from females painted with 90 ug/ml DNCB (Table). Despite the slightly larger litters from the mothers receiving the 22.5 ug/ml and 2.25 ug/ml, body weights of the 22.5 ug/ml neonates was not significicantly different from the controls or from the neonates born of mothers receiving 90 ug/ml (Figure 1).

Strikingly, however, a dose response effect was observed in the

body weight gain of the progeny, which was directly proportional to the dose of DNCB received. Those pups from mothers receiving 90 ug/ml initially at the 3rd day of pregnancy showed only a minimal reduction in body weight gain from the control ($p<0.01$ at 7 days and only $p<0.05$ at 28 days). When 90 ug/ml was applied starting on the 12th day of pregnancy, differences were either only marginally significant ($p<0.05$) at 14 days, and significant at $p<0.01$ at 21 days (Figure 1). Significant inhibition of body weight gain at the median DNCB dose (22.5 ug/ml) was seen at 7 to 28 days when the sensitizing agent was applied starting on the 3rd day of pregnancy, and at 14 to 28 days when this dose was applied starting on the 12th day (Figure 1). The severest arrest of body weight gain occurred in the pups from mothers receiving 2.25 ug/ml DNCB (for almost all time intervals from day 0 to day 28, $p<0.001$; Figure 1).

Discussion These data show that the higher the dose of DNCB, the less toxic it is on the ability to maintain and increase body weight. it must be emphasized here that non-toxic effects of DNCB also is reflected by no abortions of any type at the dose-regimens used. However, at lower doses, DNCB appears to influence some physiologic activity leading to the inability to maintain the appropriate conditions for body weight gain. Thus, the efficiency of DNCB as a therapeutic agent in HIV-infected progeny (and perhaps other individuals) may depend on how much of this agent is introduced. Within the limits of this study, it would appear that a higher dose of DNCB would be less toxic (more effective) as a

therapeutic agent.

Explanations for the reduction of BW gain with lower doses of DNCB as compared to an apparent lack of effect on BW with higher doses are not readily apparent. Perhaps the up-regulatory modulation of CMI, as demonstrated by others[6], reflects stimulation of APC including a vigorous immune action that provides beneficial physiologic conditions, while at lower doses a tolerogenic effect on APC is induced leading to a state that is not conducive to normal physiologic weight gain. This concept agrees with current opinions on dose-response effects and generation of neonatal tolerance[14,15]. Obviously our results need to be confirmed in a larger study using T-cell, cytokine, and APC factors in mothers and pups to ascertain the effects of DNCB on transplacentally exposed progeny.

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	PROGENY				MOTHERS				
Exptl Group	No. of pups	% overall survival	% pup survival[a] 3dadncb	12dadncb	No.of mothers	% BW increase[b] 3dadncb	12dadncb	% survival[c] 3dadncb	12dadncb
Control	13[d]	92	83	100	2[d]	107	109	100(1)	100(1)
90 ug	19[e]	95	100	92	3	108	109	100(1)	100(2)
22.5 ug	25[e]	94	100	94	3	99	114	100(1)	100(2)
2.25 ug	23[e]	94	100	94	3[f]	107	ND	100(1)	50(2)

[a]dncb, dinitrochlorobenzene applied topically in ug/ml; [b]Frequency of body weight (BW) increase for 1 mother only for each experimental group at each time interval of initial dncb application; [c]Survival values for the number of mothers used in each group. Numbers in parentheses indicate the number of mothers used in each experimental group; [d]Number of pups born in litters from 2 mothers; [e]Number of pups or fetuses in litters of 3 mothers; [f]After the initial application of dncb at 12 days of pregnancy at 2.25 ug/ml, one mother died after the 2nd application, and pups from the litter of 1 mother topically treated with dncb at 3 days of pregnancy were not weighed.

Table: Effect of dncb on mean litter size, survival, and maternal body weight increase

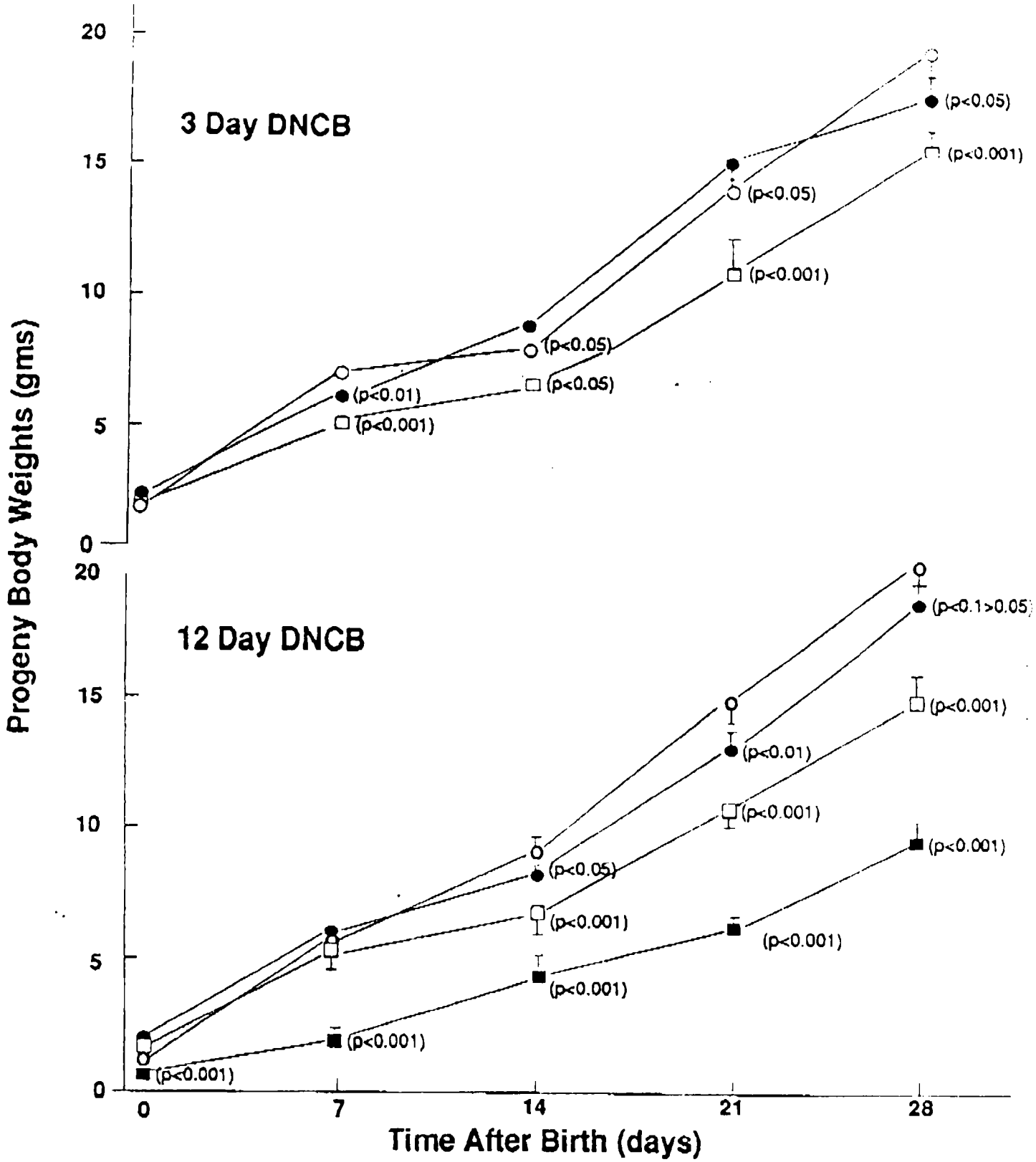

Figure 1: **Body weight (BW) changes of progeny transplacentally exposed to DNCB.** (O) Control; (●) 90 ug/ml DNCB; (□) 22.5 ug/ml DNCB; (■) 2.25 ug/ml DNCB. Each point represents 6 to 17 determinations. Since there was little change in BW for controls after gestational exposure to DNCB at 3 (six progeny) or 12 (six progeny) days, the data were pooled. Vertical lines represent standard deviations from the mean. Confidence intervals at >95% were considered significant. This is reflected by the p values at selected intervals (points) on the graphs (see text for explanation of statistical evaluations). Values for p were obtained by comparing 90 ug/ml mean BW vs control BW; 22.5 ug/ml vs 90 ug/ml, and 2.25 ug/ml vs 22.5 ug/ml.

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March 7, 2000

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Subject: Safety of DNCB

I am pleased to comment on the safety of 2,4-dinitro-1-chlorobenzene, DNCB, the common name of this classic chemical. The Chemical Abstract name is 1-chloro-2,4-dinitrobenzene.

I served as Director of Bioassay, National Cancer Institute, in the late 1960s through 1972. We tested a series of chemicals for possible carcinogenic or mutagenic effects, and worked on their likely mechanism of action, as a basis for human risk assessment.

We, and others, observed that nitrobenzene derivatives could be mutagenic in bacterial assay systems, such as the *Salmonella typhimurium* assay of Ames. In Ames test system, nitro compounds are often positive, because bacteria generate a reactive hydroxylamine, that can act as a mutagen in bacteria. Important is the fact that chemicals with a nitro group in a given position on the benzene ring are not mutagenic or carcinogenic in mammals, because in mammalian systems, the nitro group is reduced completely to a non-reactive amino group. This would apply to DNCB.

Consequently, while DNCB was weakly mutagenic in the bacterial Ames test, it is completely inert in mammalian cell systems. DNCB was also negative in our long-term carcinogenicity test in mice and rats (see *J. Environ. Path. Toxicol.* 2:325-356, 1978).

Thus, I conclude that DNCB is safe for human use, especially upon cutaneous application. The finding that DNCB stimulates the immune system and helps to control AIDS is a major discovery and, I hope, will find wide clinical application.

With all good wishes,

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Evidence That Prior Immune Dysfunction Predisposes to Human Immunodeficiency Virus Infection in Homosexual Men

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Summary: To investigate the role of host susceptibility to HIV-1 infection, we studied subsequent seroconversion in 161 individuals, initially seronegative to HIV-1, who underwent skin testing for cutaneous anergy at an index visit within a prospective study of homosexual men. There were 23 seronversions in these men by 45 months following the skin testing, yielding a crude rate of seroconversion of 14.3%. While results of purified protein derivative (PPD), *Candida*, and *Trichophyton* skin tests were not associated with subsequent course, anergy to dinitrochlorobenzene (DNCB) was predictive of subsequent seroconversion. Kaplan–Meier estimates for the risk of seroconversion during 45 months of follow-up in those men initially anergic and reactive to DNCB were 28.9 and 11.1%, respectively, yielding a relative risk of 2.6 ($p = 0.006$). The estimated relative risk was stable with adjustment by Cox regression for annual number of male sexual partners and frequency of receptive anal intercourse, and was not sensitive to various changes in the definition of seroconversion time and of eligibility criteria. These data suggest that an impaired host immune status may be associated with an increased risk of HIV-1 infection that is independent of risk of exposure to the virus, supporting earlier speculations that HIV-1 may itself be opportunistic. The notion of varying host susceptibility to infection, at least with regard to sexual transmission in homosexual men, may help to explain the frequent observation of individuals who have been repeatedly exposed to the virus and yet have remained uninfected. Key Words: Human immunodeficiency virus—Host susceptibility—Dinitrochlorobenzene.

Several studies have confirmed that the predominant risk factors for infection with human immunodeficiency virus type 1 (HIV-1) in homosexual men are an elevated number of male sex partners and an increased frequency of receptive anal intercourse (1–3). These variables act presumably as exposure factors, the former by increasing the probability of contact with an infectious partner, and the latter by providing an efficient means of transmission given such contact. The role of host factors is less clear although there is recent evidence that positive herpes serology is associated with increased host susceptibility to HIV-1 in homosexual men (4).

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While the relationship between HIV-1 infection and subsequent impairment of immune function is well recognized, it is noteworthy that the converse has also been proposed, namely that independently of the other known risk factors, prior immune dysfunction might act as a host susceptibility factor to HIV-1 infection (5). The purpose of the present study is to test the hypothesis that, among uninfected homosexual men, immune dysfunction as measured by failure to respond to certain skin test antigens (namely tuberculin, *Candida*, *Trichophyton*, and/or dinitrochlorobenzene) might be predictive of subsequent HIV-1 infection. The degree of association between various laboratory parameters and later seroconversion is also reported.

METHODS

As described previously (3,6,7), the Vancouver Lymphadenopathy–AIDS Study (VLAS) is an ongoing prospective study of over 700 homosexual men who were recruited from six general practices located in the central area of Vancouver, Canada, during the period of November 1982 to February 1984. During each visit, a questionnaire was administered, a complete physical examination performed by the subject's physician, and a blood sample drawn for immunologic and HIV-1 antibody testing. Participants may have had additional HIV-1 antibody tests between study visits at their physician's discretion. Visits were conducted on a semiannual basis until October 1986; since that time they have occurred annually, with the eighth cycle of visits nearing completion in September 1988.

As well, at the time of the first two visits, a consecutive subsample of subjects were scheduled for skin testing for cutaneous anergy. This included epicutaneous sensitization with 2,4-dinitrochlorobenzene (DNCB). DNCB was dissolved in acetone to form a solution of 2 mg/0.1 ml. A total of 0.1 ml of this solution was then applied to a 2 cm diameter circle on the skin of the upper arm using a tuberculin syringe. Each site was covered with a porous tape (Micropore) that was removed in 24 h. The patients were instructed not to wash until the tape had been removed. The sites were examined at 48 h and at 14 days. Sensitization was defined as the presence of an acute eczematous reaction at 14 days. If no sensitization reaction was present at 14 days, the patients were patch tested with DNCB (1% wt/vol) in petrolatum using an aluminum-back patch test occluded with porous tape. This patch test was read at 48 h with reactivity defined as the presence of a localized acute eczematous reaction. Patients who were nonreactive at the first visit were resubmitted to sensitization at the second set of skin tests. Reactivity to specific recall antigens was tested as well by intradermal injection of test antigens using a 1 ml tuberculin syringe. The test antigens included tuberculin (Connaught Laboratories, Willowdale, Ontario, Canada), as well as *Candida albicans* and *Trichophyton mentagrophytes* (Hollister-Stiehr, Spokane, WA, U.S.A.). The doses of antigen used were 0.1 ml for tuberculin (5 TU/ml) and 0.025 ml for *Candida* and *Trichophyton* (1,000 PNU/ml). The tests were read at 48 h, with reactivity defined by the presence of erythema and induration at least 10 mm in diameter. All skin tests were administered and interpreted by one dermatologist (W.A.M.) who did not have access to the participants' questionnaire responses or laboratory results.

HIV-1 antibody tests were performed at the Laboratory Centre for Disease Control in Ottawa, Canada using the ELISA assay with positive and equivocal results confirmed by Western blot. A small number of samples were later thawed and tested for the presence of HIV p24 core antigen using an antigen capture ELISA method (Dupont/NEN Research Products). We also measured the following laboratory parameters as previously described (8): hemoglobin, WBC, absolute lymphocyte count, IgG, IgA, IgM, C1q binding, CD4 count, CD8 count, and CD4/CD8 ratio. During the initial phase of the study, lymphocyte subsets were performed only on a random subsample of the cohort.

For the purpose of this investigation, the second visit will be referred to as the index visit and all laboratory and skin test data will be those obtained from that visit. To be eligible for the analysis, a participant was required to have been skin tested at the index visit and to have been HIV-1 seronegative at that time. Seroconversion in this group was defined by the occurrence of a positive HIV-1 antibody test result subsequent to the index visit. The date of seroconversion was estimated as the midpoint of the time interval between the last negative and first positive HIV-1 antibody test.

Baseline comparisons of subgroups defined by initial skin test status were made using the nonparametric Wilcoxon rank sum test. Time to subsequent

seroconversion starting from the date of the index visit was examined using methods of survival analysis (9). Individuals who remained persistently seronegative were considered right-censored at the time of their last HIV-1 result. The cumulative seroconversion rate in various subgroups and the mean time to seroconversion were estimated by the product-limit method of Kaplan and Meier. Seroconversion rates in different subgroups were compared by the log rank test. For comparing seroconversion in those reacting to a skin test versus those not reacting, one-sided p values were computed because of the prior hypothesis about the direction of the association (that anergy would be associated with seroconversion). In examining the association between skin test results and later seroconversion, nominal p values were adjusted by the Bonferroni method to take account of multiple hypothesis testing. After checking the adequacy of the proportional hazards assumption, Cox regression was used to estimate the relative hazard of seroconversion in various subgroups with adjustment for potential confounding variables.

The association between several laboratory measurements performed on baseline blood specimens and later seroconversion was examined using the Wilcoxon rank sum test.

RESULTS

A total of 310 men in the study were seronegative at the index visit. A total of 306 men (139 seropositive, 167 seronegative) underwent one or more skin tests at the index visit. Of the 167 individuals who were both initially seronegative and skin tested, 6 had no follow-up information available and were eliminated from the analysis, leaving a cohort of 161 eligible men. Not all of these men underwent all four skin tests; the numbers who received *Trichophyton*, tuberculin, *Candida*, and DNCB were 146, 143, 154, and 149, respectively.

A total of 23 seroconversions were observed in these 161 men during the 45 months subsequent to the index visit for a crude seroconversion rate of 14.3%. For the HIV-1 seroconverters, time to seroconversion ranged from 2 to 27 months, with an estimated mean time of 11.0 months. Among the 138 eligible men who did not seroconvert, follow-up time ranged from 4 to 48 months, with an estimated mean time of 30.5 months. The comparison of seroconversion rates among men reacting and men not reacting (anergic) to a skin test is presented for all four tests in Table 1. There was no association between anergy to *Trichophyton* or to tuberculin and later seroconversion. While there is a trend toward greater seroconversion in those anergic to *Candida* relative to those reactive, this association did not approach statistical significance.

There was, however, a strong observed association between DNCB anergy and later HIV-1 seroconversion. Among the 149 men who underwent this test, 116 responded to DNCB sensitization, while 33 (22.1%) did not and were classified as DNCB anergic. As seen in Table 1, among the 33 DNCB-anergic subjects, 9 (27.3%) subsequently seroconverted as compared to only 12 (10.3%) of 116 DNCB-reactive men. The Kaplan–Meier estimates for the 45 month seroconversion rates in the DNCB-anergic and reactive groups were 28.9 and 11.1%, respectively, with a nominal one-tailed p value of 0.006.

Figure 1 presents a comparison of the cumulative seroconversion curves for the DNCB-anergic and DNCB-reactive groups. The 9 seroconversions in

TABLE 1. *Comparison of subsequent seroconversion rates by response to skin testing at index visit in a cohort of homosexual men*

Skin test	Response	Proportion seroconverting	Kaplan–Meier estimate[a]	p Value[b]
Trichophyton	Anergic	14/106	14.2%	0.48
	Reactive	6/46	14.4%	
Tuberculin	Anergic	17/121	15.1%	0.77
	Reactive	4/22	20.0%	
Candida	Anergic	8/41	22.0%	0.13
	Reactive	14/113	13.1%	
DNCB	Anergic	9/33	28.9%	0.006
	Reactive	12/116	11.1%	

[a] Kaplan–Meier estimate for cumulative risk of seroconversion within 45 months following index visit.
[b] Based on log rank test, one-sided p value.

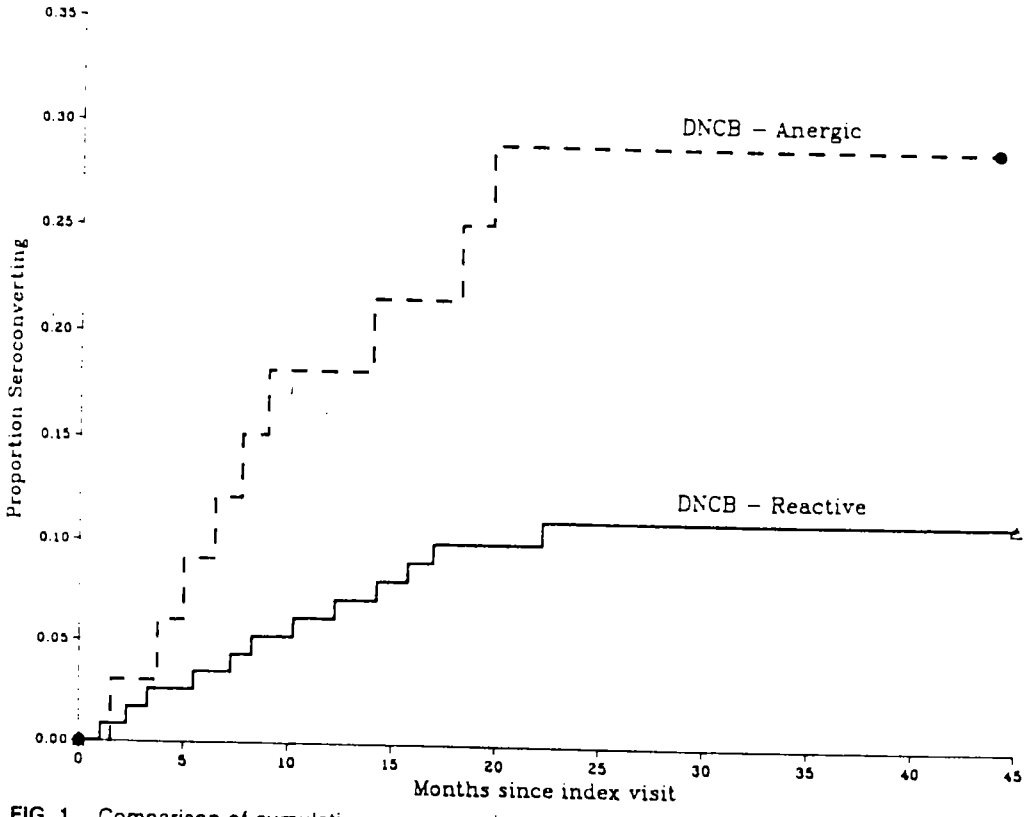

FIG. 1. Comparison of cumulative seroconversion curves for DNCB-anergic and DNCB-reactive groups.

the DNCB-anergic group occurred a mean of 10.7 months following the index visit, while the 12 seroconversions in the reactive group occurred at a mean of 11.0 months following the index visit (p = 0.95; log rank test). Among those who did not seroconvert, the follow-up times for the DNCB-anergic and -reactive subjects were similar, with mean censoring times of 29.8 and 31.1 months, respectively (p = 0.39; log rank test).

We conducted a further analysis restricted to those 140 individuals still seronegative at least 3 months after the index visit and found that the estimated relative risk of seroconversion in those DNCB anergic relative to those DNCB reactive was 2.7 (p = 0.03).

Of the nine DNCB-anergic seroconverters, frozen sera from the index visit were located in sufficient amount for p24 antigen testing for four subjects (with no serum located for one case, and insufficient serum for testing in four others). All four samples tested were negative for p24 antigen.

The men who responded to DNCB were compared to those who did not on a number of baseline characteristics measured at the time of the index visit. The results of these comparisons may be seen in Table 2. These two groups were similar with respect to age, behavioral characteristics including number of sex partners and frequency of receptive anal intercourse, and with respect to a number of laboratory parameters including lymphocyte subsets.

The Cox proportional hazards model was used to check the possibility that the observed association between DNCB anergy and later seroconversion might be due to confounding by other known risk factors for HIV-1 infection. The unadjusted relative risk from the Cox model for seroconversion in DNCB-anergic individuals relative to DNCB-reactive individuals was 2.84 (95% CI: 1.20–6.08). When adjusted for number of episodes of receptive anal intercourse with distinct partners in the preceding year, the relative risk was 2.71 (1.13–7.33). The relative risk was also stable under adjustment for all of the baseline factors listed in Table 2 (taken singly), and for the three other skin test results.

None of the laboratory parameters obtained at the index visit was associated with subsequent seroconversion in our cohort. The results of compar-

TABLE 2. *Baseline characteristics in a cohort of 149 homosexual men classified by DNCB result*

Variable	DNCB reactive			DNCB anergic			p Value[a]
	N	Mean	SD	N	Mean	SD	
Age	116	33.7	7.45	33	32.6	6.48	0.68
Partners[b]	115	6[d]	—	33	6[d]	—	0.82
Receptive anal intercourse[c]	115	3.6[d]	—	33	3.6[d]	—	0.80
Hemoglobin (g/L)	109	15.5	1.07	32	15.4	.65	0.71
WBC (per mm³)	111	6563	1842	33	6052	1583	0.16
Lymphocytes (per mm³)	110	2211	732	32	1999	794	0.07
IgG (mg/dl)	95	997	248	29	1095	358	0.53
IgA (mg/dl)	95	200	94	29	226	135	0.25
IgM (mg/dl)	95	132	52	29	137	51	0.77
C1q binding (%)	84	7.08	3.1	26	7.35	4.4	0.57
CD4 count (per mm³)	75	926	343	25	848	365	0.26
CD8 count (per mm³)	75	562	197	25	525	187	0.43
CD4/CD8 ratio	75	1.71	.55	25	1.65	.49	0.65

[a] Wilcoxon rank sum test.
[b] Number of different partners in preceding year.
[c] Number of episodes involving different partners in preceding year.
[d] Median.

isons of these parameters in seroconverters and nonconverters are presented in Table 3. Similarly, among the 157 men who were seronegative at the index visit but who were *not* skin tested, there was no association between laboratory parameters at the index visit and later seroconversion (data not shown).

The 149 eligible men who were DNCB skin tested were compared to the group of men who were seronegative at the index visit and who had subsequent follow-up but who were not skin tested (i.e., the group that was not eligible solely on the basis of not having been skin tested). The results of this comparison are presented in Table 4. The distributions of these baseline characteristics are seen to be quite similar for the two groups.

DISCUSSION

There are several possible explanations for the observed association between DNCB anergy and later seroconversion that are worthy of discussion. These include chance, bias, confounding, reverse causation, and, finally, causation.

With regard to the possibility that this association with DNCB is a chance finding, the probability that one would find an association by chance alone at a nominal significance level of 0.006 is 0.024, under the conservative assumption that the four skin tests are independent. The true significance level may be less considering that the various skin-test results are in fact correlated.

Several possible sources of bias were considered.

TABLE 3. *Comparison of laboratory measurements at the index visit in subsequent seroconverters and nonconverters in a cohort of homosexual men*

Variable	Seroconverters			Nonconverters			p Value[a]
	N	Mean	SD	N	Mean	SD	
Hemoglobin (g/L)	21	15.1	1.26	120	15.5	0.93	0.10
WBC (per mm³)	21	6386	1703	123	6456	1815	0.58
Lymphocytes (per mm³)	20	2140	915	122	2167	723	0.71
IgG (mg/dl)	20	1074	323	104	1010	270	0.30
IgA (mg/dl)	20	184	73	104	210	110	0.49
IgM (mg/dl)	20	127	46	104	135	52	0.78
C1q binding (%)	16	7.6	3.2	94	7.1	3.5	0.43
CD4 count (per mm³)	15	898	384	85	908	344	0.96
CD8 count (per mm³)	15	541	183	85	555	197	0.38
CD4/CD8 ratio	15	1.69	0.54	85	1.70	0.54	0.97

[a] Wilcoxon rank sum test.

TABLE 4. *Baseline characteristics in a cohort of 302 homosexual men classified by whether DNCB skin testing was done or not*

Variable	DNCB tested			Not DNCB tested			p Value[a]
	N	Mean	SD	N	Mean	SD	
Age	149	33.5	7.24	152	32.6	7.35	0.23
Partners[b]	148	6[d]	—	152	6[d]	—	0.14
Receptive anal intercourse[c]	148	3.6[d]	—	152	3.6[d]	—	0.28
Hemoglobin (g/L)	141	15.5	.99	147	15.4	.95	0.58
WBC (per mm³)	144	6446	1793	146	6492	2749	0.44
Lymphocytes (per mm³)	142	2164	749	149	2159	713	0.79
IgG (mg/dl)	124	1020	279	131	1017	273	0.59
IgA (mg/dl)	124	206	105	131	192	94	0.22
IgM (mg/dl)	124	133	51	131	145	60	0.26
C1q binding (%)	110	7.15	3.5	127	7.65	4.7	0.69
CD4 count (per mm³)	100	907	349	80	934	355	0.57
CD8 count (per mm³)	100	553	194	80	599	245	0.27
CD4/CD8 ratio	100	1.70	.53	80	1.67	.63	0.40

[a] Wilcoxon rank sum test.
[b] Number of different partners in preceding year.
[c] Number of episodes involving different partners in preceding year.
[d] Median.

There was no evidence of any difference in the distributions of censoring times for the nonconverters in the anergic and reactive groups, suggesting that more intensive follow-up of the anergic group did not take place. Nor was there a difference in the frequency of visits or in the time interval between visits for the two groups. The method of calculating the seroconversion time as the mid-time point between the last negative test and the first positive test might be questioned. The analysis was therefore repeated assuming seroconversion immediately after the last negative HIV-1 serology date, with the results essentially unchanged.

Another possible explanation for the observed association is confounding by other risk factors for HIV-1 exposure. If the same risk factors that give rise to HIV-1 exposure also entail a risk of anergy to DNCB, then one might anticipate the observed association. For example, DNCB anergy due to multiple exposures to other viruses and antigens might be more frequent in those men with an elevated number of sex partners and/or an increased frequency of anal receptive intercourse. Since the latter are also the predominant risk factors for HIV-1 infection, an association between DNCB anergy and subsequent seroconversion would be expected. However, since the strength of the association between DNCB status and later seroconversion (as measured by relative risk in the Cox model) is essentially unchanged with adjustment for other variables in the data set, including the known predictors of HIV-1 infection, it is unlikely that confounding by these variables could account for the observed association. Misclassification in the measurement of confounding variables may seriously impair the ability to adjust for the effect of a confounding variable (10). However, even with misclassification, one would expect to see an attenuation of the association if confounding is present and this was not seen in our data. Confounding by a factor outside of the currently established causal pathway, however, cannot be excluded.

We must also consider the possibility that some of the participants, although HIV-1 antibody negative, might have already been infected with HIV-1 at the time of the index visit. If HIV-1 infection gave rise to anergy to DNCB even before seroconversion had occurred, then the observed association could be due to the fact that anergy is an effect of HIV-1 infection rather than a cause, i.e., reverse causation. There are several considerations against this explanation, however. First, when the analysis is restricted to those individuals still seronegative 3 months after the skin test was performed (N = 140), the estimated relative risk is 2.7, with the strength of the association showing no trend towards weakening, although the statistical significance is less because of the smaller sample size. Second, baseline laboratory parameters including IgG and C1q binding are similar for the DNCB-reactive and the DNCB-anergic groups whereas one would expect increases in the latter group if early HIV infection were present. Third, mean time to seroconversion was similar in DNCB-anergic and DNCB-reactive seroconverters. Thus, there is good evidence that the relative risk is stable over time,

not initially higher as one would expect if the DNCB-anergic group were already infected at baseline. An additional minor piece of evidence is that p24 antigen testing was negative at the index visit in all four DNCB-anergic seroconverters in whom testing was carried out.

Finally, there remains the hypothesis that given equal exposure to HIV, homosexual men who are DNCB-anergic are more susceptible to infection than are DNCB-reactive men. This explanation implies that while HIV-1 exposure is certainly necessary for infection to develop, factors not directly related to exposure are important in the causal chain, at least in some cases of infection. Well before the original discovery and characterization of HIV-1 and the clarification of its role in AIDS, numerous mechanisms were proposed as possible causes of the immunodeficiency seen in AIDS (5). Hypotheses considered at that time included antigen overload resulting from repeated exposures to microbial pathogens (11), the effect of infection with immunosuppressive viruses such as Epstein–Barr virus (EBV) (12) and cytomegalovirus (CMV) (13), and immunosuppression due to exposure to alloantigens expressed on sperm (14) or transfused lymphocytes (15). With the discovery of HIV-1, however, the contribution of such factors in the pathology of AIDS was discounted, although their role as potential cofactors in disease outcome among HIV-1-infected individuals is presently under investigation in prospective studies. While such investigations may reveal a role for these as cofactors once HIV-1 infection has been established, there have been few recent reports that implicate predisposing immune factors in host susceptibility to HIV-1 infection. Previous studies in homosexual men and in hemophiliacs have shown evidence of immune dysfunction that was apparently not accounted for by HIV-1 infection (5,16–21). Tsoukas et al. (18) found that 6 of 14 hemophiliacs showed evidence of functional cellular immunodeficiency despite being HIV-1 antibody negative. Another group of seronegative hemophiliacs was shown by Ludlam et al. (19) to have altered lymphocyte subsets. Madhok et al. (20) found that 9 of 19 clinically severe hemophiliacs had an abnormal response to DNCB testing. This was not related to HIV-1 seropositivity, but an inverse relation between DNCB response and previous clotting factor exposure was detected, suggesting that an agent or agents in clotting factor other than HIV-1 may have been responsible for the immune defect.

Of the four skin tests that were performed in our study, only abnormal reactivity to DNCB sensitization was found to be predictive of subsequent infection, whereas anergy to recall antigens was not. This would suggest that it is something particular to the type of response being tested by DNCB that is associated with the increased risk. One evident difference between DNCB and the other three skin tests is that the latter measure the recall of a previous exposure and hence serve as a measure both of exposure and of immune memory to these antigens. DNCB, on the other hand, is not an environmental antigen and most individuals would not be expected to have been sensitized. The inability of some of the seronegative individuals in our study to respond to a secondary challenge with DNCB must therefore reflect either a genetic predisposition to DNCB anergy, or a more fundamental difficulty in mounting primary immune responses. One would predict on this basis that DNCB-anergic subjects would also have problems mounting immune responses to other antigens against which they had not previously been sensitized. HIV-1 may represent such a new antigen, fulfilling to a degree the role of "opportunistic infection" as originally proposed by Levy and Ziegler (5).

Questions naturally arise as to the extent of impairment of cell-mediated immunity in HIV-1-negative at-risk individuals and to factors predisposing to such impairment. External causes such as exposure to immunosuppressive viruses such as EBV or CMV could be responsible (12,13). Exposure to sperm alloantigens or other immunosuppressive factors may be another avenue by which such a defect may arise (21,22). We did not find an association in our data between anergy to DNCB and sexual risk factors that would be likely to increase the risk of these latter exposures. The number of individuals, however, may not have been sufficient to reveal a subtle association. On the other hand, exposure to clotting factor has been associated with DNCB anergy in hemophiliacs (19). Shearer and Hurtenbach (22) reported that a number of cellular immune functions were disrupted in mice by prior injections of whole sperm. Cytotoxic T-cell (CTL) responses against hapten-modified self- and alloantigens as well as mixed lymphocyte reactivity were reduced. These effects seemed to be antigen nonspecific but the onset of impairment of CTL function was detected earlier with hapten-modified cell antigens than with alloantigens. Findings of reduced responses to influenza virus-infected self-cells

among HIV-1 seronegative homosexual men were also reported by Shearer et al. (23).

The nature of the population studied deserves comment. To be eligible for this analysis, an individual had to have remained uninfected through the second cycle of our study so that men at highest risk who were infected early in the epidemic and hence already seropositive at the time of study enrollment were not eligible. The present results are thus based on a relatively low-risk group of homosexual men, beyond which the results should not be generalized. Indeed, it is tempting to speculate that if host susceptibility is truly a factor in HIV-1 infection, it is likely to have its greatest effect in low-risk homosexual men such as we have studied, where exposure may be infrequent and where host factors may be more likely to tip the balance in favor of the establishment of infection (21).

The established list of determinants of HIV-1 transmission includes the presence of the virus in a body fluid or medium, the concentration and viability of the virus in that fluid or medium, and access of the fluid or medium to a portal of entry. Our data suggest that host susceptibility should also be included as a determinant of infection following exposure to the virus, at least with regard to sexual transmission among homosexual men. As noted by Klatzmann and Gluckman (21), the notion of varying host susceptibility to infection may help to explain the frequent observation of individuals in various settings who are known to have been repeatedly exposed to the virus and yet have remained uninfected (3,24–27). However, we must caution that although we may have identified a role for host susceptibility as a determinant of HIV-1 infection, there are no data at present to support the concept of *absolute* resistance to infection in any population. There is nothing in the present data, for example, to suggest that a healthy individual with an intact immune system cannot become infected after even a single sexual encounter. Thus, the implications of these data for current public health and educational initiatives are limited. Moreover, if it is the case that the risk factors that promote host susceptibility among seronegative individuals are also risk factors for exposure to HIV-1, then the potential for further reduction of HIV-1 transmission beyond that offered by modification of exposure factors alone is also limited. Further study of the causes and extent of immune impairment in seronegative at-risk individuals is needed to determine if modifiable factors exist that might offer the potential for further reductions in the transmission of HIV-1 by lowering host susceptibility to infection.

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DEIN

FROM: DR. JEANETTE ROBINSON
EXECUTIVE DIRECTOR

SUBJECT: THE WHITE HOUSE COMMISION ON COMPLIMENTARY AND ALTERNATIVE MEDICINE POLICY

TO: MR. STEPHEN C. GROFT
EXECUTIVE DIRECTOR

Thank you very much for letting us provide input to your commision.

DR. JEANETTE ROBINSON

NOTE: 3 pages including cover

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August 27, 2000

Mr. Stephen C. Groft
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Bethesda, MD 20817-7707

SUBJECT: The White House Commission on complementary and Alternative Medicine Policy

Dear Mr. Groft,

It is with great enthusiasm that our organization provide input regarding the importance of alternative and complimentary medicines as we continue to learn and develop new products and explore the benefits of both.

Our think piece in concern with cardiovascular group and individualized therapies and the significant benefits derived as a preventative as well as post therapy to cardiovascular fitness. Endurance Therapy (aerobic) has many benefits when conducted by highly trained individuals to work with special needs populations, as well as under served populations.

There should be more research and funding to study the benefits of preventative medicine as it pertains to endurance therapy to sustain improved cardiovascular systems when enhanced with antioxidant supplementation.

In addition, we are also concerned with how some over-the-counter, or multi-level nutrition distributors, provide testimonies to potential customers, from other distributors in order to promote their products to people who are unaware of certain herbal remedies, herbal/vitamin blends and other forms of herbal combinations, which promises to give the customer increased health by just taking a pill. There must be a mechanism in place to educate the public about the latest products that are being sold through direct mail or other forms of distribution.

Our organization has conducted extensive research on the effects of various herbs, such as Kola Nut, MaHung, and herbs combined with St. John's Wart. These herbal combinations can cause harmful, if not deadly side effects.

Instead of enhancing the cardiovascular system during exercise and providing a sense of balance to the central nervous system, it causes heart palpitations, depression, irritability and severe mood swings. In cases as early as 1991, the combination of Kola Nut and MaHung eventually causes several deaths. Therefore, the known benefits of some herbs can be diminished due to abuse, and wrong combinations, and the recommended dosage can be too potent.

How can we gain more control of herbal combinations and vitamin combinations, as well as other energy enhancing or healing substances, which are disseminated widely to the public? Do we need more warning labels or more education for the consumer? Could the council publish a newsletter regarding these issues?

We definitely agree that the choice of future medicine is alternative medicine. But we must look at various alternative remedies being purchased from distributors everyday. This market must be studies because most people who use these therapies choose multi-level marketing companies before they would go to a natural practitioner.

Please keep our organization informed of any new studies, research or how we can also assist your council. You may reach the undersigned at (937) 275-2536 or via email as shown above.

Sincerely,

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EXECUTIVE DIRECTOR

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Kingsbury, Doris (NCCAM)

From: Adam Burke, PhD [aburke@pacbell.net]
Sent: Wednesday, October 11, 2000 12:27 PM
To: Whccamp
Subject: Re: Written comments


WHCCAMP.Burke
RTF


WHCCAMP.Burke W5

Hi:

I have attached as Word 5 and Rich Text Format documents. Also, enclosed is text below. Thanks.

Adam Burke

--
Adam Burke, PhD, MPH
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> From: Whccamp <Whccamp@OD.NIH.GOV>
> Date: Wed, 11 Oct 2000 07:22:05 -0400
> To: "'Adam Burke, PhD'" <aburke@pacbell.net>
> Subject: RE: Written comments
>
> The attachment file would not open.
>
>> -----Original Message-----
>> From: Adam Burke, PhD [SMTP:aburke@pacbell.net]
>> Sent: Monday, October 09, 2000 12:49 PM
>> To: Whccamp
>> Subject: Written comments
>>
>> Greetings:
>>
>> I was a panelist at the September 8th meeting in San Francisco. Enclosed
>> is
>> a copy of my written comments. Could you please let me know that you
>> received them and that you were able to open them. They are in Word 98.
>> Thanks, I enjoyed the opportunity to share some ideas with the panel.
>> Good
>> luck! I think your efforts are very important.
>>
>> Adam Burke
>>
>> --
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White House Commission on CAM Policy
September 8, 2000
San Francisco, California

Panel member:
Adam Burke, PhD, MPH, LAc
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What is Alternative About Alternative Medicine?

Ten years ago my friend Suzan was diagnosed with aggressive lung cancer. She was 45 years old. I remember the day I accompanied her on a visit to a major Bay Area cancer treatment center. The oncologist was polite and to the point. She told us in the three minute interview that statistically there was no hope for survival. The doctor advised no treatment. The interview was over. That was very, very disheartening. I then took Suzan to see a highly respected herbalist I had apprenticed with in San Francisco's Chinatown. We sat down with him and talked about politics and life in America. He pointed proudly to his naturalization papers. After about twenty minutes of conversation he began to discuss the treatment plan with my friend. He said to her, "I cannot promise that we will save your life. No one can do that. But let's see what we can do."

That experience changed my life, and shaped my beliefs about alternative medicine. It was the difference between treating a human being as a statistical probability and treating someone as a person with fears and hopes and dreams. As a social psychologist, health educator and acupuncturist it made me begin to reflect on the question, "What is alternative about alternative medicine?" That is what brings me here this morning. My concern is that in our efforts to understand this phenomena we may completely miss the point, the most important point, by virtue of our theoretical perspectives and research methods. If we focus on finding the most potent ingredient in the medicinal herb, or the western physiological equivalent of qi so that we can avoid such arcane concepts, we may prevent ourselves from looking more deeply into a rich field of possibilities.

We need to ask ourselves, "What makes alternative medicine alternative?" I would suggest that it is not the needles, not the dong quai or ginseng, not the exact position of the hands in the energy healing process. Perhaps the key to alternative medicine is that it is outside the established system, so it is more personal, slower, more caring. Perhaps because these therapies are lower in the power hierarchy there is less arrogance. Perhaps it is the beliefs and lifestyles of the practitioners that serve as models for their clients. The challenge for us is that we bring to this exploration a particular world view. Our cosmologies, our scientific theories, our models of causation, can blind us from seeing new realities. We may not know what questions to ask, because we do not even know what is possible, or we may not be willing to consider the options. For those who understand the scientific process, and who are honest, it is clear that our choice of research methods affects the data that drives the theories we come to cherish. We need to be careful in our research efforts, to ask ourselves what are we really doing, what are we really looking for.

If we approach this exploration in the wrong way we may miss the whole point of alternative medicine. Actually, as the East is changing so rapidly, if we do this research in the wrong way we may risk losing this knowledge for all time. We need to avoid the temptation of short-term gain, like some medicinal mercenary going in and chopping down the only remaining stand of

an exotic curative plant or trapping the last of some endangered animal for its prized essence. Rather, we must proceed like ecologists who recognize the enormous value of this precious, intact area. The goal is not to find the one most potent ingredient, but to understand the interrelationship of things that allow this healing medicine to work.

To this end the following ideas are offered as key considerations:

1. Ask Bigger Questions

It would be productive to explore the bigger question, "What is alternative about alternative medicine?" Is it the herb that cures cancer, or is it something about the relationship with the herbalist, or is it both? Alternative medicine is more than alternative techniques, or even philosophies. It is perhaps about the different lifestyles and personalities of the practitioners, the evocation of hope, and many, many other factors. It is imperative that we concurrently explore the psychosocial, biological, behavioral, environmental and spiritual aspects of this process. The deeper questions that arise in this inquiry are, "What is healing, and how do we heal?" If we can approach this endeavor with those broader questions in our vision we will serve both the understanding of alternative medicine and the general efficacy of all medical and healing practices.

2. Explore Research Methods

Consider the choice of research methods. The methods we select will consequently shape the data we gather and the theories we come to believe. We need to explore other research methods in addition to randomized controlled clinical studies.

3. Encourage Curiosity

We need to enter this enterprise with an open mind, with true curiosity, and with a belief that someone else may know more than us. When I began acupuncture school I had few preconceptions or biases. I was very intrigued by this poetic description of the body and healing and allowed myself the opportunity to be steeped in that world view for many years. It was a gift and I was changed by the process. Perhaps we need to approach this task more like anthropologists. It is important that we come to a better understanding of the plethora of approaches before embarking too aggressively on efficacy research. We need to avoid coming to premature closure on what constitutes effective practice, as we do not want to confirm a few methods and then close the door on other options. The Flexner Report in the early 1900's shut down certain healing perspectives and effectively obstructed exploration and discovery in the United States for a century. Also, as in all worlds, alternative medicine has powerful factions that would like their methods to be pre-eminent socially and economically. Efforts must be made to keep explorations open.

4. Promote Collaboration

We must bring the alternative healing community more directly into the research process. This may prove challenging as the harassment of alternative practitioners continues today. Thirty years ago in California, one of our esteemed acupuncturists, Miriam Lee, was severely persecuted right here in the Bay Area as an example to other acupuncturists. Fortunately, our then-Governor, Jerry Brown, was enlightened enough to help legalize acupuncture in California which changed history forever. Even ten years ago I personally had to keep certain aspects of my alternative background out of my resume if I wanted to be hired for academic or other types of work. Academia would not see you as serious or rigorous if you had strong personal interests in alternative issues. So alternative people will not necessarily come running to help in this process, but their input must be solicited. The allopathic medical community still acts as the primary selection committee, determining what will be on the menu and who will be invited to the table. This limitation of perspective can only lead to limited outcomes. It will make us proceed without sufficient understanding. That is a dangerous position from which to make determinations of what shall remain of the ancient healing cultures. The potential hegemony resulting from that approach will cost us dearly, causing us to miss the real magic

and ending up with a few new techniques to add to the same old paradigm. To promote collaboration we specifically need to:

a. Involve more alternative practitioners in the planning and research process. That will require thoughtful selection, and training, and empowerment. We have to think of it like any other type of diversity program. We need honest and adequate representation. That may require grass roots outreach and training in the ideas and practices of research. We should also involve more active collaborations between established research institutions and CAM clinics and practitioners who have records of success. This will require more funding to create an adequate national CAM research infrastructure. Funding, collaboration and representative expertise are critically important elements of success.

b. Reduce the number of MDs on the review panels. Recently I did two CDC review panels for proposed CAM research projects. One common problem with the applicant grants was that their CAM panels were often so heavily MD-oriented. I suspect the applicants felt content as they included CAMish MDs, but we must at least acknowledge the possibility that an MD by virtue of family background, years of undergraduate and graduate training, career and social status, type of personality, and other factors, is a different type of person with a different world view. To deny that is to truly not understand a key aspect of this issue. If we want to perpetuate the same mythology then we need to just fill the panel with MDs, CAMish or not.

5. Evaluate the Preventive Lifestyle Orientation

We need to recognize that the alternative health movement is not necessarily about alternative medicine. Perhaps it is more accurately a program of alternative health practices, preventive lifestyle strategies. This week in class I asked my students how many of them see an allopathic physician when they are well. No one raised a hand. Then I asked how many get massage, take herbs, do yoga, or meditate when they are well. The vast majority raised hands. The alternative philosophies encourage health-oriented lifestyles. That is a foundation of the eastern perspective. The ancient medical practices have to do with how we live our lives, moment to moment. They are profoundly preventive and self-directed. We can benefit by building that lifestyle model into our health care systems. Exploring that perspective will be essential if we hope to truly understand and benefit from this alternative healing phenomenon.

6. Update Education and Research Training

We need to inform and educate consumers in an honest and unbiased way about the value of alternative therapies. More importantly, we need to begin to train students, at an undergraduate and graduate level, in the concepts and practices of alternative health care. Students should be exposed to research methods suitable for exploring this area, and work in broadly based cross-disciplinary teams to understand the complex interactions of alternative healing practices. San Francisco State University has been pioneering in this area for 20 years and has seen a growing tide of student interest. A new type of health professional is emerging and public education needs to be a proactive participant in helping to build that future.

7. Cross Fertilize and Promote Quality

We should bring this same process of inquiry to allopathic medicine, examining what healing is and what heals. Through a concurrent exploration of these deeper questions in both allopathic and alternative medicine we can catalyze the enrichment of all major traditions. In the end this will produce a significantly greater understanding of human health, healing and well-being, and lay the foundation for a solid future of high quality and continuous improvement.

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October 8, 2000

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Dear Dr. Jonas:

Thank you for allowing me the opportunity to present testimony on behalf of the chiropractic research community at last week's WHCCAMP meeting in Washington, D.C. I appreciate the enormity of the task of the Commission in organizing the vast amount of information put forth at just one of its many meetings scheduled; by the same token, it was undoubtedly a challenge for me to try to fit all of what I believe are cross-cutting barriers and possible solutions in CAM research written in my testimony into 10 minutes of oral presentation.

As promised, I am documenting the publication of a research network of medical and chiropractic physicians that we talked about on Friday. The enclosed paper [**Attachment 1**] is a pilot study, representing a reduced sample of health providers from both professions. For the short-term outcomes [pain severity, functional disability, sensory and affective pain quality] in chronic low-back pain patients, greater improvements were seen in those individuals under chiropractic care. A second paper by the same investigative team [in press at the same journal] representing the patients of 111 medical and 60 chiropractic providers, suggests more equivalent outcomes at 6 and 12 months with an advantage seen in patients with radiating pain below the knee after adjusting for baseline differences. Regarding your question about the status of the chiropractic research network overall, I trust that Bill Meeker has been able to provide you with information about the Palmer Center's research efforts.

I also wish to call to your attention an unfortunate development that has occurred regarding the Veterans' Administration pertaining to the provision of chiropractic services. From the enclosed attachments, I hope that you will recognize that the VA appears to have resisted implenting the spirit if not the letter of the Millenium Healthcare Act of 1999, which was to overcome earlier restrictions by the VA in providing chiropractic services to veterans.

Attachment 2A depicts the initial recognition by the House of the findings of recent outcomes research in low-back pain. This endorsement ultimately was signed into law in November of last year [**Attachment 2B**], indicating that "doctors of chiropractic should be fully engaged and integrated into primary physician status with the Veterans Health Administration." Despite the chiropractic community's provision of complete documentation and a meeting with Thomas V. Holohan and a task force from the Veterans' Administration

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on February 24 of this year, the VA did not return a policy recommendation congruent with what appears to be a mandate for direct access and scope of practice to the extent allowed by state licensure laws. Rather, it appeared to do everything it could to preserve its previous policy barring both direct access and primary care functions on the part of chiropractors.

As indicated by **Attachment 2C**, the VA through its House Committee, presented an "alternative" proposal at the very last minute to the leading chiropractic organizations, calling upon the VA to "develop" but not implement the policy originally mandated in 1999. Incredibly, the VA added insult to injury by insisting that due to time constraints, no further modifications of the proposal would be allowed. As shown in the narrative provided by the attachment, there was little alternative for chiropractic organizations than to reject the proposal outright and attempt to start over.

The most charitable thing that I can say regarding this response by the VA is that it is disingenuous. For an organization to turn around and attempt to demonstrate its interest in CAM before the Commission by enumerating research projects in the hundreds as it did last Friday appears to me to be egregiously hypocritical, given its track record with chiropractic services. I deeply regret having to discredit one of the invitees before the Commission, but when a major policy of thwarting the implementation of CAM practices is pursued by a party which attempts to profess otherwise takes place, I become justifiably perturbed.

I do hope that you will be able to take this information into consideration in your deliberations, in addition to my annotated testimony which was distributed to the Commission last week. Please do not hesitate to call on me for expanding upon any of the documentation which I have provided thus far or to answer any additional questions that may arise.

Sincerely yours,

Anthony L. Rosner, Ph.D.
Director of Research and Education

Enclosures

xc: James S. Gordon, M.D.
Stephen C. Groft, Pharm.D.

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Patient Characteristics, Practice Activities, and One-Month Outcomes for Chronic, Recurrent Low-Back Pain Treated by Chiropractors and Family Medicine Physicians: A Practice-Based Feasibility Study

Joanne Nyiendo, PhD,[a] Mitchell Haas, DC,[b] and Peter Goodwin, MD[c]

ABSTRACT

Background: Chronic low-back pain is a significant public health problem for which few therapies are supported by predictable outcomes. In this report, practice activities and 1-month outcomes data are presented for 93 chiropractic patients and 45 medical patients with chronic, recurrent low-back pain.

Design: A prospective, observational, community-based feasibility study involving chiropractors and family medicine physicians.

Setting: Forty private chiropractic clinics, the outpatient clinic of the Department of Family Medicine at Oregon Health Sciences University, and 5 other Portland area family medicine clinics.

Outcomes Measures: The main outcome measures were pain severity, functional disability, sensory and affective pain quality at 1 month, and patient satisfaction assessed at 7 to 10 days and at 1 month.

Results: Although differences were noted in age, sex, education, and employment, the patients were closely matched at baseline with respect to frequency, severity, and type of low-back pain and the psychosocial dimensions of general health. The treatment of choice for chiropractors was spinal manipulation and physical therapy modalities; for medical physicians antiinflammatory agents were most frequently used. Chiropractic patients averaged 4 visits, and medical patients averaged 1 visit. On average, chiropractic patients showed improvement across all outcomes: 31% change in pain severity, 29% in functional disability, 36% in sensory pain quality, and 57% in affective pain quality. Medical patients showed minimal improvement in pain severity (6%) and functional disability (1%) and showed deterioration in the sensory (29%) and affective (26%) dimensions of pain quality. Satisfaction scores were higher for chiropractic patients. Outcomes for medical patients were heavily dependent on psychosocial status at baseline.

Conclusion: Patients with chronic low-back pain treated by chiropractors show greater improvement and satisfaction at 1 month than patients treated by family physicians. Nonclinical factors may play an important role in patient progress. Findings from the Health Resources and Services Administration-funded project will include a report on the influence of practice activities, including more frequent visits by chiropractic patients, on the clinical course of low-back pain and patient outcomes. (J Manipulative Physiol Ther 2000;23:239-45).

Key Indexing Terms: Low-Back Pain; Outcome Assessment (health care); Chiropractic; Family Practice; Feasibility Studies

INTRODUCTION

Back pain, especially low-back pain, continues to be a major problem affecting the health care system in this country. At any given point in time, up to 20% of adults report that they have symptoms of back pain.[1] It is the second leading cause of all physician visits in the United States.[2] Although seldom life-threatening, it is a major cause of functional disability, representing one fourth of all disabling work injuries.[3] Frymoyer and Cats-Baril[1] suggest that total costs for low-back pain disorders may have been as high as $75 to $100 billion in 1990. The cost burden is disproportionate, with 75% to 90% of the costs attributable to only 5% to 10% of patients with low-back pain. These are individuals for whom back pain has become chronic and disabling.[4,5] There is little evidence of any significant abatement of this public health problem, in spite of the fact that the proportion of health care resources devoted to low-back pain conditions continues to increase.[6,7]

The causes of back pain are multifactorial. The natural history of low-back pain syndromes,[4] the presence of confounding factors (physical, psychological, social, and economic),[8] and the heterogeneity of these conditions[9] present a difficult situation for the clinician. In most cases a definitive diagnosis is not possible.[4,10] This uncertainty has resulted in a variety of treatment practices that are largely empirical. Clinicians are unable to predict with confidence which patients are most likely to benefit from care and under which circumstances.

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In spite of the tremendous burden patients with chronic low-back pain put on the health care system, there is a paucity of data on treatment effectiveness for this patient group.[4-11] Federally-sponsored practice guidelines have been developed only for acute low-back problems in adults.[12] For chronic low-back conditions, there are many issues of clinical management for which questions remain unanswered; among them are the following: How do differences in the treatment processes relate to variations in outcomes among different types of practices and providers? What is the relative benefit of different care strategies?

In an attempt to begin to answer these questions, we conducted a prospective, observational, practice-based feasibility study involving chiropractors and family practice physicians. Our strategy was to collect data on patient outcomes and physician practice activities. The focus of this article is a single profile: patients with chronic recurrent ambulatory low-back pain.

METHODS

Clinic Settings and Physician Participants

Physician participants numbered 33 medical physicians and 45 chiropractors. The study sites included 40 private chiropractic clinics, 80% located in the Portland metropolitan area; the outpatient clinic of the Department of Family Medicine at Oregon Health Sciences University in Portland; and 5 other Portland-area family medicine clinics. Clinics were oriented to the protocol and logistics of data collection during a 3-month period in late 1992 by the staff of the Center for Outcomes Studies. Methods were established for integrating the study into the clinic routine with minimal disruption and without jeopardy to either patient care services or the integrity of the study. Data were collected on 797 patients with acute and chronic low-back pain over a 6-month period in 1993. This article describes the subgroup of patients with chronic low-back pain.

Subjects

Patients were eligible for the study if they were 18 years or older, had a primary complaint of low-back pain, and had not been previously treated by a participating physician or a physician of the same provider type for the current episode. Patients were excluded if any of the following applied: lumbar fracture, instability, malignancy, or infection; or other sources of low-back pain of nonmechanical origin, such as referred pain of organic origin.

For the purpose of this article, "chronic" was defined as an episode duration of 6 weeks or longer. Although the work group on the Classification of Chronic Pain[13] suggested that the term "chronic" be used at 3 months after the index episode, the Report of the Quebec Task Force[4] suggested a cut-off at 7 weeks, identifying a period between 1 week and 7 weeks as a "sub-acute period." Recent longitudinal studies on the evolution of chronic back pain lend support to this shift closer to the time of initial injury.[14] Recurrent episodes were defined as discreet episodes of low-back pain separated by 6 weeks or more of no low-back pain.

Patients were informed of the protocol and that their responses would be kept confidential. The study was approved by the institutional review boards of Western States Chiropractic College and Oregon Health Sciences University. All patients were required to sign an informed consent form before enrollment.

Study Protocol

Both patients and physicians completed a questionnaire on the first visit. Patient follow-up included a telephone interview at 7 to 10 days and a mailed questionnaire at 1 month and 3 months. Physicians were required to complete a follow-up questionnaire at all subsequent visits.

Patient Questionnaires

The patient baseline was administered in the waiting room before the clinical encounter and consisted of 6 parts. Sociodemographic information was requested. A condition-specific questionnaire established the state of the patient's low-back condition before treatment.[15] A 100-mm visual analogue scale (VAS) was used to measure pain severity[16] and the short-form McGill Pain Questionnaire (MPQ) was included to document pain quality.[17] The Revised Oswestry Low-Back Pain Questionnaire (RODQ) was used to assess limitations in functional activities.[18] Eight elements of general health status were measured with the Medical Outcomes Study 36-item Questionnaire (SF-36).[19] Three questions were appended to the SF-36 to screen for depression.[20] The time required to complete the patient baseline ranged from 15 to 30 minutes.

The 9-question telephone interview assessed patient satisfaction by use of a 5-point ordinal scale. The questionnaire was modeled after the Cherkin and MacCornack satisfaction questionnaire[21] and was conducted at 7 to 10 days. The 1-month patient follow-up was mailed with a postage-paid, self-addressed return envelope. It included a condition-specific questionnaire, VAS, MPQ, and RODQ.

Physician Questionnaire

The physician's record sheet was a 14-item structured, double-sided, self-administered questionnaire that took less than 1 minute to finish. It was completed either during or immediately after the clinical encounter. Physicians were asked to provide data on clinical impression and patient management. Information requested on practice activities included treatment procedures used, type and region of adjustment (chiropractic patients only); type and dose of drugs used (medical patients only); and type and frequency of use of ancillary procedures, such as heat or cold application, electrotherapy, and ultrasonography. The physician follow-up form was identical to the baseline physician's record sheet.

RESULTS

The patients with chronic, recurrent low-back pain included in this report were a small subgroup (17.3%) of the total enrolled sample; 93 chiropractic patients and 45 medical patients comprised this patient profile. Recruitment rates

Table 1. *Patient characteristics*

	Chiropractic (n = 93) (%)	Family practice (n = 45) (%)
Mean age (SD)	40.4 (13.4)	48.1 (15.8)
Sex		
Male	45.2	28.9
Female	54.8	71.1
Race/ethnicity		
Black	2.2	4.4
White, non-hispanic	85.9	77.8
Native American	9.8	13.3
All other	2.2	4.4
Education		
<High school graduate	7.6	11.1
High school graduate	17.2	28.9
Some college	37.6	40
College graduate/graduate work	37.7	20
Employment (outside home)		
Full time	64.5	36.4
Part time	14	11.4
None	21.5	52.3
Job-related injury	16.9	15.9

were assessed by chart audit 4 months into the study at 2 medical and 4 chiropractic clinics; 75% to 88% of eligible chiropractic and 42% to 74% of eligible medical patients were asked to participate. Of those recruited, 88% to 93% of the chiropractic patients and 56% to 78% of the medical patients were enrolled. The satisfaction interview at 7 to 10 days was completed by 76% of the chiropractic patients and 78% of the medical patients. Response rates at the 1-month follow-up for chiropractic and medical patients were as follows: MPQ, 53% and 51%; and RODQ and VAS, 47% and 38%.

Patient Baseline Characteristics

Table 1 displays the patient characteristics within the 2 provider groups. Differences were seen with regard to age, sex, and employment status. Compared to chiropractic patients, family practice patients tended to be older, female, less educated, and less likely to be employed outside the home. General health status profiles for family practice patients at baseline showed slightly greater physical impairment, bodily pain, and poorer general health (Figure 1). On the other hand, scores assessing psychosocial health were essentially identical in the 2 patient groups. Although acute depression was reported with nearly equal frequency (chiropractic, 43%; medical, 45%), chronic depression of 2 years duration (dysthymia) was more prevalent in patients seen by medical physicians, 31% versus 14%.

The frequency of previous low-back pain episodes in the past year was similar. Low-back pain severity in the week immediately preceding the index visit was more often reported to be severe or extremely severe (35.7% vs 23.6%) by medical patients. Seventy-nine percent of family practice patients and 60% of chiropractic patients reported symptoms of sciatica (data not shown).

Condition-specific outcomes

The means and standard deviations for pain severity (VAS), pain quality (MPQ), and functional disability

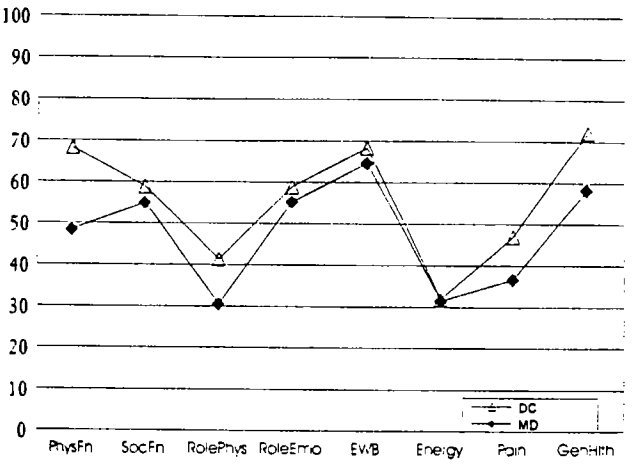

Fig 1. *Health status profiles for medical and chiropractic patients at enrollment. For all dimensions, optimal health is represented by a score of 100.* PhysFn, *Physical functioning (limitations on physical activities such as lifting, climbing, walking);* SocFn, *limitations on social activities (visiting with friends, relatives) as a result of physical or emotional health problems;* RolePhys, *limitations on work or other activities of daily living as a result of limitations in physical functioning;* RoleEmo, *limitations on work or other activities of daily living as a result of mental/emotional health;* EWB, *emotional well-being (mental health);* Energy, *energy/fatigue level;* Pain, *degree of interference with normal work as a result of pain;* GenHlth, *perception of health, in general.*

(RODQ) are presented in Table 2. The MPQ characterized 2 separate dimensions of pain quality: sensory (eg, temporal, spatial, thermal, and pressure); and affective (eg, tension, fear, and autonomic properties).[17] Considerable variation in the primary outcome measures is indicated by the large standard deviations. On average, family practice patients were characterized by greater impairment (higher scores). However, baseline scores were nearly identical for the subset of medical and chiropractic patients who submitted their 1-month follow-up questionnaire. Chiropractic patients showed greater improvement at 1 month on the 2 MPQ dimensions and on the VAS. Family practice patients actually regressed 26% to 29% on the MPQ dimensions. Although 6% improvement was noted for medical patients on the VAS, the improvement for chiropractic patients was 5 times greater, at 31%. Chiropractic patients also showed less functional disability at 1 month. The baseline difference in RODQ scores (7.5) is not likely to have had a clinically important effect on the 1-month follow-up scores.

Patient satisfaction

Table 3 presents data on patient satisfaction obtained by telephone interview at 7 to 10 days. With one exception, satisfaction was higher for patients attending chiropractors. Notable differences (≥20%) were found for statements related to the patient's assessment of his/her physician's concern and for statements related to the patient's own level of comfort.

Table 4 depicts the 3 aspects of patient satisfaction that were measured at 1 month. Chiropractic patients expressed greater satisfaction regarding information and treatment pro-

Table 2. *Baseline, follow-up scores, and mean change (Δ) in primary outcome measures at 1 month*

	Chiropractic		Family practice	
	Mean/SD	%	Mean/SD	%
VAS				
Baseline: all patients (n = 87, 33)	42.0 (21.4)		43.9 (24.8)	
Baseline: patients with follow-up (n = 44, 17)	42.5 (21.4)		41.9 (26.4)	
1 Month	29.4 (23.8)		39.4 (26.4)	
Absolute Δ	−13.1 (23.8)		−2.5 (24.0)	
Relative Δ		−31%		−6%
RODQ				
Baseline: all patients (n = 88, 42)	38.5 (14.9)		48.1(15.0)	
Baseline: patients with follow-up (n = 44, 17)	38.9 (13.8)		46.5 (14.9)	
1 Month	27.6 (18.6)		45.8 (19.1)	
Absolute Δ	−11.3 (15.4)		−.6 (9.8)	
Relative Δ		−29%		−1%
MPQ				
Sensory				
Baseline: all patients (n = 90, 43)	7.1 (5.6)		8.7 (7.9)	
Baseline: patients with follow-up (n = 49, 23)	7.3 (5.0)		7.3 (7.3)	
1 Month	5.0 (5.6)		9.4 (7.8)	
Absolute Δ	−2.6 (5.5)		2.1 (6.7)	
Relative Δ		−36%		29%
Affective				
Baseline: all patients (n = 90, 43)	1.9 (2.6)		2.7 (3.2)	
Baseline, patients with follow-up (n = 49, 23)	2.3 (2.7)		2.3 (3.0)	
1 Month	1.0 (1.8)		2.8 (3.2)	
Absolute Δ	−1.3 (2.2)		.6 (2.4)	
Relative Δ		−57%		26%

Higher scores at baseline and 1 month represent impairment. Hence, a negative score for absolute Δ or relative Δ represents improvement.
Absolute Δ, One-month score − baseline score; *Relative Δ*, mean absolute Δ/mean baseline score.

Table 3. *Patient satisfaction at 7 to 10 days**

Patients who strongly agree or agree with statement	Chiropractic (n = 71) (%)	Family practice (n = 35) (%)
Patient's assessment of physician's concern		
My doctor spend adequate time listening to my description of the pain	97.2	77.1
My doctor understood my concerns about the cause of my pain	98.6	74.3
My doctor seemed to agree that my pain was real	100	85.7
Patient's impression of physician's confidence		
My doctor seemed confident that the diagnosis she/he gave me was correct	92.9	93.3
My doctor seemed confident that the treatment she/he recommended would work	94.3	79.3
My doctor seemed comfortable dealing with my back pain	98.6	87.9
Patient's own level of comfort		
My doctor gave me sufficient information about the cause of my pain	78.6	65.7
I knew what to do to take care of my back after the visit with my doctor	95.7	71.9
I feel confident that the treatment my doctor recommended will work	81.4	53.4

*Statements were scored on a scale of 1 to 5, with 1 = strongly disagree and 5 = strongly agree.

vided. The difference between provider groups was most marked for the question involving satisfaction with overall medical care (chiropractic, 90%; medical, 52%). Chiropractic patients also reported greater improvement at 1 month (Table 4) as measured by a subjective assessment question. A higher proportion of chiropractic patients (56% vs 13%) reported that their low-back pain was better or much better, whereas nearly one third of medical patients reported their low-back pain was worse or much worse.

Physician practice activities

Radiographs were obtained on the first visit with nearly equal frequency by medical physicians and chiropractors (24.4% and 24.7%). Antiinflammatory drugs were the most frequently prescribed medication by family practice physicians (42%). Muscle relaxants were used infrequently (6%). Six patients (13%) were referred to physical therapy; only 2 patients actually complied with this recommendation. In 3 of 4 chiropractic cases, the principal adjustive method was manual, high-velocity, low-amplitude manipulation. Chiropractic patients were more likely to receive full spine adjustment (61%) and less likely to receive adjustment only in the lumbopelvic region (39%). Physical therapy modalities were frequently used, with massage and trigger-point therapy used most often. Electrotherapy, heat, and ultrasonography were used in one third of cases. For both medical and chiropractic patients, ancillary procedures most often included an exercise plan and postural advice. Lumbar supports were recommended for 5% of chiropractic patients and for none of the medical patients. Bed rest was rarely advised in either group. The mean number of office visits for chiropractic patients was 4.3 (SD = 3.0); for medical patients, 1.1 (SD = 3.0).

Correlations

Correlations were calculated to explore the association of 1-month outcomes with baseline health status (Table 5). The highlights are discussed below. Spearman's rank correlation coefficient (rho) is reported rather than Pearson's correlation because of the slight skewness of the data and sensitivity of Pearson's r to outliers in small samples.

For both patient groups, poorer baseline status was associated with poorer follow-up status for pain severity and functional disability. The correlation between baseline affective pain quality and follow-up RODQ was fairly strong for the medical cohort (r = .64) but trivial for the chiropractic cohort (r = .05). Additionally, cohort differences were noted on the 4 dimensions of the health status questionnaire that measure psychosocial attributes. Poorer health at baseline on these dimensions was associated with greater impairment at 1 month. The outcomes for chiropractic patients seemed to be less affected by initial status for mental health, limitations on activities of daily living caused by mental health dysfunction, and social function. Chiropractic patients with impaired status in the psychosocial dimensions were just as likely to show

Table 4. *Satisfaction and patient improvement (subjective) at 1 month**

	Chiropractic (n = 50) (%)	Family practice (n = 23) (%)
Patients' satisfaction at 1 month		
How satisfied with information given about condition?		
Very/somewhat satisfied	82	61.9
Neither satisfied nor dissatisfied	12	19
Somewhat/very dissatisfied	6	19
How satisfied with treatment for condition?		
Very/somewhat satisfied	89.8	65
Neither satisfied nor dissatisfied	2	10
Somewhat/very dissatisfied	8.1	25
How satisfied with overall medical care?		
Very/somewhat satisfied	89.8	52.4
Neither satisfied nor dissatisfied	6.1	23.8
Somewhat/very dissatisfied	4	23.8
Subjective assessment of improvement		
Compared to the last questionnaire, is low-back pain better/worse?		
Much better/better	56	13
A little better/about the same	30	52.1
A little worse/much worse	14	34.8

*Satisfaction with information, treatment and overall medical care were scored on a scale of 1 to 5, with 1 = very satisfied and 5 = very dissatisfied. Subjective assessment was scored on a scale of 1 to 6, with 1 = much better and 6 = much worse.

Table 5. *Correlations of baseline health status with primary outcome measures at 1 month**

	One-month follow-up			
	VAS		RODQ	
	DC (n = 44)	MD (n = 21)	DC (n = 45)	MD (n = 22)
Baseline status				
Pain severity: VAS	**.44**	**.52**	**.49**	.2
Functional disability: RODQ	.36	**.52**	**.52**	**.85**
Pain quality: sensory	.39	.39	.12	.35
Pain quality: affective	.3	.36	.05	**.64**
General health status				
Physical function	**−.46**	−.33	**−.54**	**−.69**
Role-physical	−.26	−.37	**−.48**	**−.64**
Role-emotional	−.02	−.12	−.03	**−.57**
Energy/fatigue	−.28	−.15	−.3	**−.58**
Emotional well-being	−.12	−.18	−.11	**−.55**
Social function	−.14	−.29	−.12	**−.62**
Pain	−.29	−.17	**−.57**	**−.62**
General health	−.01	−.2	−.07	−.09

*Better baseline status was found to be associated with better follow-up status for all measures. Correlations greater than .40 in magnitude are in bold-face. All correlations for general health status are negative because of the directionality of the scales.

improvement on the primary outcome measures as patients with better psychosocial health.

Correlations between 1-month follow-up scores and 1-month satisfaction with treatment were unremarkable. Within the chiropractic cohort, there was little evidence of any relationship between treatment outcomes and satisfaction with information, treatment provided, or overall care. On the other hand, an unexpected association was found between medical patients' satisfaction and change scores for VAS and Oswestry: the greater the improvement, the more dissatisfied family practice patients were with the treatment received.

DISCUSSION

A variety of procedures are available to treat low-back pain, but, with few exceptions, it is not known which patients are likely to benefit from which treatment and under which circumstances. For chronic low-back conditions, there is little consensus regarding the most appropriate method of treatment. Patients may be receiving less than optimal care. The current climate of health care reform, with its emphasis on patient outcomes and physician accountability, provides a unique opportunity for practice-based researchers. This study is important because it represents an effort by chiropractors and family medicine physicians to systematically measure patient outcomes. The preliminary data obtained on patient characteristics, practice patterns, and patient outcomes revealed some findings of interest that suggest directions for hypothesis development and future research.

Patient Characteristics

The 2 patient cohorts presented some sociodemographic differences (age, sex, education, employment). At baseline, patients of family practice physicians scored more poorly with respect to overall health status and ability to perform the usual tasks of daily living (eg, walking, climbing stairs, lifting). They also reported slightly more bodily pain. These findings may be a consequence of the 8-year mean age difference between the cohorts. The effect of these potential confounders on outcomes is being explored in the long-term study.

Practice Activities

Although research published in both the chiropractic and medical literature has repeatedly shown radiographic findings to be poorly correlated with low-back pain,[22-26] in this study of chronic low-back pain, 1 in 4 patients underwent radiography. The duration of the problem beyond 1 month,[27,28] coupled with physician uncertainty,[29] may have influenced the decision to obtain radiographic films, a decision made with nearly equal frequency by the 2 physician types.

The therapy of choice for participating family physicians was antiinflammatory drugs (eg, ibuprofen). The findings from the first study were consistent with the literature that describes medical treatments as typically including drugs, physical therapy, bed rest, and exercise regimens.[30] Ninety-six percent of chiropractic patients received spinal manipulative therapy. The greater use of full-spine adjustment may be a consequence of chiropractors' view of the spine and locomotor system as an integrated unit. As low-back pain becomes chronic, local problems may cause adaptations and compensation, which potentially lead to secondary problems in other regions of the spine.[31] The extensive use of ancillary procedures is consistent with the literature that reports that physical therapy modalities and exercise regi-

Attachment 2B:

LEGISLATIVE HISTORY: SECTION 303 OF VETERANS' MILLENNIUM HEALTHCARE ACT OF 1999 (PUBLIC LAW 106-117)

For the past year, the American Chiropractic Association (ACA) and the Association of Chiropractic Colleges (ACC) have worked together with Congress to reintroduce the idea of assimilating chiropractic healthcare services into the Department of Veterans Affairs (DVA) healthcare system. This legislative effort began on behalf of veterans in need of chiropractic services—those who are eligible for medical care benefits under Chapter 17 of Title 38, United States Code (U.S.C.).

The Veterans' Millennium Healthcare Act was signed into law by President Clinton on November 30, 1999 (Public Law 106-117). Among other things, it included a provision requiring the Department of Veterans Affairs to develop a policy with regard to chiropractic care in the DVA healthcare system. More specifically, Section 303 of the Act requires that "Within 120 days after the enactment of this Act, the Under Secretary of Health, after consultation with chiropractors, shall establish a policy for VHA regarding the role of chiropractic treatment in the care of veterans under Chapter 17, Title 38 U.S.C."

The Committee Report accompanying the House version of the Veterans' Millennium Healthcare Act (House Report 106-237) clarifies the committee's intent in carrying out Section 303, which was ultimately modified and agreed to in conference with the Senate. The language strongly supports chiropractic healthcare services for veterans, citing both scientific journals and studies that demonstrate the benefits of chiropractic. The Report cites an earlier effort by the former Veterans Administration to study chiropractic in the VA as too restrictive in the scope of chiropractic methods applied to veterans. It also states that the program reached too few of the veterans for which it was designed. The chiropractic healthcare profession concurs with the thrust of the Report language that the policy established under the new legislation will be sufficiently broad in both scope of practice and outreach to veterans around the country, particularly in rural and medically underserved areas.

Finally, the Report states, and the chiropractic healthcare profession agrees, that doctors of chiropractic should be fully engaged and integrated into primary physician status with the Veterans Health Administration to work with DVA physicians and other medical personnel in the development and implementation of the chiropractic treatment policy that will be set forth under Section 303.

Attachment 2C:

VA

Subj: Fwd: VA news bulletin
Date: Fri, 22 Sep 2000 5:35:02 PM Eastern Daylight Time
From: Needtk
To: Dc doc1234, REVDC3, EMadcharo@nemic.com, Drs DK Bac, MikePedigo
BCC: RosnerFCER

It is virtually impossible to imagine a more irresponsible action than has been taken by the WCA regarding an effort by the chiropractic profession to obtain full rights under the VA legislation. How outrageous and distructive an act toward chiropractors and their patients everywhere, even those chiropractors who are so shortsighted as to be ignorant of the harm they are causing. We can only hope that there is an appropriate reaction by the profession to this terrible activity by WCA.

Forwarded Message:

Subj: VA news bulletin
Date: Fri, 22 Sep 2000 4:56:51 PM Eastern Daylight Time
From: Jay Witter <JWITTER@amerchiro.org>

The following is a news bulletin provided by the American Chiropractic Association regarding VA legislation.

FOR MORE INFORMATION, CALL:
ACA's Department of Government Relations
(800) 986-4636

FOR IMMEDIATE RELEASE: September 22, 2000

VETERANS COMMITTEE STAFF PROPOSAL DROPPED FROM LEGISLATION AFTER ACA, ACC, AND ICA REJECT MEASURE AS INADEQUATE

ARLINGTON, VA -- An amendment regarding chiropractic care for veterans prepared by staff of the House Veterans Affairs Committee and due to be inserted in legislation pending before the full U.S. House of Representatives on Thursday, Sept. 20, was dropped from inclusion in the overall measure after the ACA and other groups rejected the staff-authored proposal as being woefully "inadequate."

Preparation of the congressional staff-authored proposal came after ACA and the Association of Chiropractic Colleges (ACC) urged the House Veterans Affairs Committee to include a "full scope and direct access" chiropractic provision in H.R. 5109 (the Department of Veterans Affairs Health Care Personnel Act of 2000) when that bill was "marked-up" by the House VA Committee on September 13. At the committee's mark-up session, several members of the committee indicated their support for including a chiropractic-related amendment in the bill--provided acceptable legislative language could be agreed upon--prior to a vote on the measure by the full House of Representatives.

Seizing the opportunity to advance a pro-chiropractic provision in the bill,

headed for "fast track" approval by the House of Representatives, ACA, ACC and the International Chiropractors Association (ICA) submitted a detailed and jointly developed legislative proposal to the committee. The heart of the proposal included provisions that would have mandated that the Department of Veterans Affairs provide chiropractic care on a "direct access" basis, and allow doctors of chiropractic to practice their "full scope" of practice within the VA health care system, as allowed by state law. Under current VA policy, chiropractic care, which is rarely provided as a VA benefit, can only be obtained after first receiving a medical doctor's referral, and chiropractors are effectively limited to only treating "low back pain."

In response to the profession's "direct access and full scope" proposal, the House VA Committee staff prepared "alternative" legislative language, which would have required the VA to develop, but not fully implement, a program to improve access to chiropractic care within the VA. However, the staff's "alternative" proposal was only provided to ACA, ACC and ICA for review late in the afternoon of September 19, barely 18 hours before the bill was scheduled to come to the floor of the House of Representatives for a vote. Additionally, when the "alternative" proposal was provided for review, a senior committee staff representative informed ACA that due to time constraints no further modifications to the "alternative" proposal could be considered, and that the organizations representing the chiropractic profession had the choice of "taking it or leaving it." The ACA, ACC and ICA found the "alternative" proposal to be deficient in a number of ways and unanimously and forcefully rejected it. Afterward, plans by the House VA Committee to attach the staff-authored proposal to H.R. 5109 were dropped and the bill passed the full House without any chiropractic provision attached. The House bill will be married to similar legislation that has already passed the Senate, and the combined bill is expected to become law this year.

"Had we been willing to accept a grossly inferior proposal--one that would not have guaranteed access to chiropractic care in the VA--we could have achieved the inclusion of some type of chiropractic provision in the bill that passed the House," said Garrett F. Cuneo, ACA executive vice president. "Quite frankly, though, even if the staff's alternative proposal constituted a modest step forward for us--which is questionable--we weren't about to be hustled into compromising on the key issues of full scope and direct access," added Mr. Cuneo.

According to ACA Board Chairman, Dr. Michael Flynn, the association will undertake a major campaign to secure a full scope and direct access provision in the next Congress. "We're going to aggressively press our concerns with Congress until we're victorious and veterans receive the chiropractic care they need and deserve. That means we're going to have to fight back the VA bureaucrats who are totally opposed to us, win over the support of key committee staffers who aren't convinced we really need direct access, and increase our influence with key members of Congress that serve on the veterans committees in both the House and Senate. Although that won't be easy, it can be done, and we're committed to getting it done."

Many members of Congress shared ACA's disappointment that a meaningful chiropractic provision was included in H.R. 5109.

During the debate of H.R. 5109 on the House floor (televised by C-SPAN), Congressman Bob Filner (D-CA) stated, "Now I do have one disappointment in this bill, that despite a strong sentiment in the full Committee on Veterans' Affairs to move a chiropractic health care benefit amendment in this bill, we are apparently unable to reach an agreement to introduce direct access, full scope of practice chiropractic care into the VA health care system in this year."

Filner added "I know this is not a simple issue, and I know the gentleman

from Florida (Rep. Stearns) is as vitally concerned about this as I am ... and I hope that we put a chiropractic health care provision that is meaningful at the top of our committee's agenda next year so that our veterans can have direct access to this important benefit as quickly as possible."

Congressman Cliff Stearns (R-FL), chairman of the House VA subcommittee on health, also voiced his support for including a chiropractic benefit in the VA health care system. During an interview with C-SPAN after the vote, Congressman Stearns indicated his willingness to develop a strong chiropractic provision and help pass it the next Congress.

Subj: **Fwd: ACA: Lawmakers Criticize Veterans Affairs Witnesses On...**
Date: 10/7/00 2:21:42 PM Eastern Daylight Time
From: Needtk
To: Dc doc1234, REVDC3, EMadcharo@ncmic.com
To: MikePedigo
BCC: RosnerFCER

Forwarded Message:
Subj: **ACA: Lawmakers Criticize Veterans Affairs Witnesses On...**
Date: 10/5/00 4:34:00 PM Eastern Daylight Time
From: AOL News

ACA: Lawmakers Criticize Veterans Affairs Witnesses On Inadequate Chiropractic Policy

Medical Lobby Assails Efforts to Expand Access

To Chiropractic Care

WASHINGTON, Oct. 5 /PRNewswire/ -- The American Chiropractic Association (ACA) and other chiropractic groups testified in favor of direct access to chiropractic and full scope of services for veterans Tuesday during a hearing before the House Committee on Veterans Affairs Subcommittee on Health.

During the hearing, congressional lawmakers sharply criticized officials from the U.S. Department of Veterans Affairs (VA) for that agency's failure to take aggressive action to ensure that veterans are provided with chiropractic care.

"Congress, as early as 1978, authorized VA to provide chiropractic services to eligible veterans," Rep. Cliff Stearns (R-FL), subcommittee chairman, said during the hearing on chiropractic services in the VA. "But over the period of its existence, VA has never employed its first chiropractor as a VA staff practitioner in this professional field; has never developed, without prodding from Congress, any meaningful policy on chiropractic care; and until this hearing, has never had to defend its decisions to severely restrict or deny chiropractic care to veterans.

"In this member's opinion," Stearns continued, "if a health care service is licensed and fully legitimate in all 50 states and abroad, if millions of Americans are willingly paying for this service every day, if health insurers -- and even the federal Medicare program -- approve reimbursements for the service as a routine activity of doing business ... then VA needs to better articulate why VA seems to deny these services to eligible veterans."

Stearns' comments were echoed by other members of the committee who were present at the hearing, including Representatives Bob Filner (D-CA) and Luis Gutierrez (D-IL).

While the VA was harshly criticized by members of Congress, prominent medical organizations, including the American Medical Association (AMA) and the American Osteopathic Association (AOA) attempted to block efforts by the ACA and other chiropractic groups that were pushing for legislation that would mandate access to chiropractic on a "direct access" and "full scope of practice" basis.

In response, the ACA and other chiropractic groups pointed out that chiropractic adjustments have been proven effective for the nation's military personnel. Reports from the Department of Defense Chiropractic Health Care Demonstration Project (CHCDP), recently completed at military treatment facilities across the country, show that chiropractic care not only reduced disability and improved patient satisfaction, but also could potentially save 199,000 work days per year for the DOD. "I believe the benefits of chiropractic care will continue to be proven with the addition of chiropractic services in the military health care system," said ACA representative Rick McMichael, DC, a member of the CHCDP Oversight Committee.

"It should be noted that doctors of chiropractic are licensed and regulated in all 50 states as independently practicing health care professionals," Dr. McMichael continued. "All of these jurisdictions recognize chiropractors' rights and responsibilities to

serve as first- contact, portal-of-entry providers. As such, doctors of chiropractic possess the diagnostic skills necessary to differentiate health conditions that are amenable to their management from those conditions that require referral or co-management with another professional." Dr. McMichael pointed to the extensive academic and clinical training doctors of chiropractic receive through accredited chiropractic colleges across the country - training that includes the use of diagnostics and therapeutics.

Also testifying on behalf of the chiropractic profession were representatives of the Association of Chiropractic Colleges (ACC) and the International Chiropractors Association (ICA).

The hearing came just days after an amendment regarding chiropractic care for veterans prepared by staff of the House Veterans Affairs Committee and due to be inserted in legislation pending before the full U.S. House of Representatives was dropped from inclusion in the overall measure after the ACA and other groups rejected the staff-authored proposal as being woefully "inadequate." Preparation of the congressional staff-authored proposal came after the ACA, ACC and ICA urged the House Veterans Affairs Committee to include a "full scope and direct access" chiropractic provision in H.R. 5109 (the Department of Veterans Affairs Health Care Personnel Act of 2000) when that bill was "marked-up" by the House VA Committee on September 13. At the committee's mark-up session, several members of the committee indicated their support for including a chiropractic-related amendment in the bill -- provided acceptable legislative language could be agreed upon -- prior to a vote on the measure by the full House of Representatives.

The American Chiropractic Association (ACA) is the largest chiropractic organization in the country, representing nearly 20,000 members. Chiropractic is the third largest doctoral-level health care profession in the western world after medicine and dentistry. In the United States, the governments of all states license and regulate doctors of chiropractic as independently practicing health care professionals. The major treatment applied by doctors of chiropractic is spinal adjustment or manipulation to correct a subluxation.

SOURCE American Chiropractic Association

CO: American Chiropractic Association; U.S. Department of Veterans Affairs

ST: District of Columbia

IN: HEA

SU: EXE LEG

10/05/2000 16:32 EDT http://www.prnewswire.com

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December 7, 2000

James S.Gordon, M.D.
Chairperson
White House Commission on Complementary
and Alternative Medicine Policy
6701 Rockledge Drive, Suite 1010, MS-7707
Bethesda, MD 20817-7707

Dear Dr. Gordon:

I spoke before your panel in Seattle on October 31, 2000, requesting that the healthcare model that is finally proposed by your commission be broad enough to allow room for people to work with kinesiology as a primary tool without having to have a license to diagnose.

Practitioners using Applied Kinesiology need to have a license to diagnose. They may be chiropractors, naturopathic physicians, medical doctors, dentists, etc. However, a majority of the people using kinesiology around the world are using Touch for Health, Educational Kinesiology, Applied Physiology, Health Kinesiology, Biokinesiology, Wellness Kinesiology, Kinesionics, SIPS, etc. Most of us using these kinesiologies do not have degrees to diagnose nor are we interested in being able to do so. We are operating within an educational bioenergetic model. The muscle testing is a tool that we use to determine how the body is out of balance, what is needed to restore balance, and to confirm when that balance has been achieved. In Brain Gym we primarily use movements to bring about that balance, naturopaths (not naturopathic physicians) will use herbs, foods and supplements, in Touch for Health and many other kinesiologies we use acupressure, reflexes for the lymph and vascular systems, foods and nutrients, emotions, sounds and color. We are all interested in balancing the individual's mind/body system and recognize that we do not need to make a diagnosis in order to achieve that objective.

In January of this year I was one of about 24 top kinesiologists from around the world, including developers of at least nine of the most well-known kinesiologies, who met in Malibu, California, to discuss how best to develop kinesiology into the 21st century. It took us almost three days to come up with professional and lay definitions of kinesiology to describe what is the essence of what we all do. I enclose these two definitions for your perusal.

During the questioning after my presentation you asked me if I could get for you some of the research that documented the effectiveness of Brain Gym. I contacted the Educational Kinesiology Foundation for the enclosed research materials. They were very happy to make them available to you. As you would appreciate, Brain Gym is very different from much of what has been used for children with special needs, slower learners, children and adults with ADD and ADHD, etc. However, there is ample research showing that a significant number of these people can benefit from Brain Gym.

As a Brain Gym instructor, I am excited to be using simple non-invasive methods such as muscle testing and body awareness to be making a positive difference in peoples' lives. Perhaps it is not surprising that one of our major concerns is that organized groups with lobbies — massage therapists, chiropractors, naturopathic physicians, the medical profession, psychologists, etc. — not be allowed to encourage legislation to squeeze us out of the field in order to prevent "perceived competition."

This is very common to find preexisting groups attempting to quash, or corral the new kid on the block. As an example of this, in Washington state the massage board is attempting to force all people who use Touch for Health to become licensed massage practitioners. Which of course benefits all the massage schools with their 500+ hour programs.

Anything your commission can do to help prevent such future nonsense would be greatly appreciated.

For any questions arising out of what I have written, please feel free to contact me at the phone number listed above. For any questions pertaining to the Educational Kinesiology research I would direct you to:

The Educational Kinesiology Foundation
1691 Spinnaker Drive, Suite 105B
Ventura, CA 93001

805-658-7942
800-356-7109
Fax: 805-650-0524

e-mail: edukfd@earthlink.net
www.braingym.org

I was very impressed with the panel and the way in which you conducted the meeting. I wish you well with the rest of your assignment and may the powers that be feel receptive to your final report(s).

Kindest regards,

Wayne Topping

Wayne Topping, PhD, LMT
Founder, Wellness Kinesiology
Touch for Health Faculty Member

Kinesiology

Kinesiology is a non-invasive method using muscle feedback and body awareness that can help you reduce stress and pain, improve performance at school, work and home, in sports, in relationships, and promotes health and well-being.

Leadership Vision 2000, Malibu, California, January 14, 2000

Professional Definition of Kinesiology

An educational bioenergetic model using muscle testing/monitoring as a primary tool to obtain feedback from the whole being's innate wisdom to allow self regulation for their highest good.

Leadership Vision 2000, Malibu, California, January 12, 2000

The research studies summarized herein were submitted to the Educational Kinesiology Foundation in experimental or annotated design. Financial contributions to the Foundation are sought and support the ongoing editing, summarizing, or translating that makes possible this and other publications on the effectiveness of the Edu-K work.

A Chronology of Annotated Research Study Summaries in the Field of Educational Kinesiology, 1981 to Present

Selected Papers Pertaining to Educational Kinesiology

Educational Kinesiology, movement, and sensory integration: a review of recent, relevant, neuroscientific literature. Susan J. Diamond, Ph.D. ©1999.
A rare and recent academic effort to link peer-reviewed research findings and concepts from numerous disciplines, including neurophysiology, into an inclusive frame work for a scientific understanding of Educational Kinesiology as a method of benefit to educators. This study includes information on the nervous system; reflexes; hemispheric organization for vision, hearing, and laterality; emotion and asymmetry; and sensory modalities. *28 pages. References, bound with the following two papers, available for $25 US (including postage). Contact:*

Susan Diamond, Ph.D.
(604) 541-9329 (fax)
diamonds@intergate.bc.ca
13768 32 Avenue
Surrey, BC Canada V4P2B8

The ADHD controversy: drugs, labels, and stifled potential. Joan Spalding. ©1997. Published in *Brain Gym® Journal*, Volume XI, No 3, 1997.
A history of ADHD and review of the literature; discussion of the use of the drug Ritalin with ADHD; the politics and results of labeling children; educational alternatives, including Brain Gym.

NLP and the brain: some issue areas, findings, and hypotheses. Susan J. Diamond, Ph.D. ©1996.
A discussion of "process therapies" including tentative links with neural and physiological factors that could explain why these therapies work. Review of research on state-dependent learning, stress and emotion, the role of lateralization and asymmetry in perception, brain organization and handedness, attention, memory, language, hearing, disassociation, etc. *46 pages. Contact:*

Susan Diamond, Ph.D.
(604) 541-9329 (fax)
diamonds@intergate.bc.ca
13768 32 Avenue
Surrey, BC Canada V4P2B8

Brain Gym® literature review and study design proposal. Susan J. Diamond, Ph.D. ©1996.
This paper, a research study design project and a literature review, relates the impact of movement on learning to some possible theoretical foundations and accepted theories of stress, exercise, etc. Section one brings in relevant concepts from neuropsychology on anxiety; asymmetry; exercise, arousal, and attention; "state models"; neurological soft signs (behavioral impairments), etc. Includes a useful review of information from relevant fields. *Prepared in 1996 for doctoral work at*

the University of Victoria, BC Canada. Section two offers a review of research on Educational Kinesiology; a review of related research on the relationship between movement and academic learning; development of a proposed research study to expand previous studies; a proposed Brain Gym research program. 58 pages. Contact:

Susan Diamond, Ph.D.
(604) 541-9329 (fax)
diamonds@intergate.bc.ca
13768 32 Avenue
Surrey, BC Canada V4P2B8

Movement or medication? The alleviation of ADD. Dr. Anka Koelman. ©1994 and 1995. Published in *Brain Gym® Journal*, Volume XI, No 3, 1997.
A summary of two of Dr. Koelman's articles on ADD, reviewing the symptoms of ADD and discussing effective alternatives, including Educational Kinesiology, Neuro-Linguistic Programming, and Superlearning.

Experimental Research

Group I: Publications on True Experimental Research Designs

The Effect of PACE on self-reported anxiety and performance in first-year nursing students. Jan Irving, Ph.D., R.N, © 1995. Published in *Brain Gym® Journal*, Volume X, No 1, 1996.
This multiple baseline design was completed with twenty-seven first-year nursing students, using three separate groups as controls during the different phases of the nine-week study. The study measured the effects of four Brain Gym activities, making up a six-minute sequence known as the PACE process, on weekly assessments of self-reported anxiety and performance on fourteen technical-motor skill tests. The PACE group experienced a 69.5 percent reduction in self-reported anxiety and an 18.7 percent increase in performance on skill tests, as compared to continued self-reporting of high anxiety and higher failure rate in the control groups not using PACE. *Copies available for $35 US (including postage, within the United States). Contact:*

Jan Irving, Ph.D., R.N.
(503) 581-8926 (fax)
esse@ncn.com
1555 Kent Street, NW
Salem, Oregon 97304-3605

Effect of Educational Kinesiology upon simple and four-choice response times. Guruchiter Kaur Khalsa and Josie M. Sifft. © 1988. Published in *Brain Gym® Magazine*, Volume II, No. 3, 1988.
This study completed with university students compared a control group with two experimental groups, one using only Brain Gym activities, and the other experiencing Dennison Laterality Repatterning and the Brain Gym activities. The results indicated that the Edu-K groups were superior to the control group in their response time to a four-choice visual light display. The repatterned group improved by twice the amount of the Brain Gym-only group.

Effect of Educational Kinesiology upon simple response times and choice response times. Josie M. Sifft, Ph.D. and G.C.K. Khalsa, ©1991. Published in *Perceptual and Motor Skills*, 73, 1011-1015.

Publications on Experimental Research

Educational Kinesiology: empowering students and athletes through movement. Josie M. Sifft, Ph.D. ©1990.
An overview of Educational Kinesiology, explanation of some of the Brain Gym® activities, and review of the research to date. *Available from Education Resources Information Center, or on microfiche (ERIC Document Reproduction Series No. ED 320891*

Effect of Educational Kinesiology on static balance of learning disabled students. Published in *Perceptual and Motor Skills*, 67, 51-54 Khalsa, Guruchiter Kaur, Morris, G.S. Don, and Sifft, Josie M., Ph.D. ©1988.

The effects of Educational Kinesiology upon the static balance of learning disabled boys and girls. G.C.K. Khalsa, and Josie M. Sifft, Ph.D. ©1988. *Available from Educational Resources Information Center, or on microfiche (ERIC Document Reproduction Series No. ED 289835).*

Effect of Educational Kinesiology on response times of learning disabled students. Guruchiter Kaur Khalsa and Josie M. Sifft. Published in *Brain Gym® Magazine* as "Effect of Brain Gym on Response Time," Volume IV, No 2, ©1990.
This study was completed with 52 children selected from Special Day classes. The Brain Gym group performed a sequence of activities, while the control group engaged in random movements for about seven minutes. All children were tested for visual response time before and after the movement activities. The results indicated that those children exposed to the Brain Gym movements improved on the response-time task, while those in the control group did not.

Group II: Publications on Quasi-Experimental Research Designs

Brain Gym® and its effect on reading abilities. Cecilia K. Freeman, M.ED. ©2000.
This study was completed using a nonequivalent control group design. A total of 205 students were assigned to either the Brain Gym or the control group. Throughout the 1998-99 school year, 12 teachers incorporated Brain Gym in the classroom curricula so that the students and teachers did a minimum of 15 minutes of Brain Gym per day. Equal samples of students were randomly selected for the Brain Gym group and the control group who did not use Brain Gym, and their test scores were compared. The results indicated that those children in the Brain Gym group improved their reading abilities, as measured by a standardized test, twice as much as did those in the control group.

The study can be used in these ways:
It can be replicated. 2. It is a model that can be taken to an administrator with a request that Brain Gym be introduced into the school. 3. It includes innovative suggestions on how a classroom teacher can use the Brain Gym activities in the classroom. *Copies are available for $20 plus $3.20 shipping in US (a 30% discount, plus postage, for orders of 10 or more). 58 pages. Contact:*

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Effects of Educational Kinesiology upon the static balance of learning disabled boys and girls. Guruchiter Kaur Khalsa and Josie M. Sifft. ©1988.
This study was completed with 60 elementary students who were classified as learning disabled. An equal number of boys and girls were divided into three groups: repatterned Edu-K, Edu-K movement, and a control. The results indicated that the Repatterned Edu-K group showed a greater improvement in static balance than the Edu-K movement group, who in turn performed better than the control group. The findings also suggest that Edu-K can be used effectively in a coeducational setting.

Group III: Publications on Pre-experimental Research Designs

Individual Brain Gym® work in a learning-assessment lab. Susan J. Stewart, Ph.D. ©1998. Published in *Brain Gym® Journal* as "Research: Brain Gym in British Columbia Public Schools," Volume XIII, No 1, 1999.
In 1995, Susan Stewart, an educational consultant, worked on a project with children in a public school in British Columbia. The purpose of the project was to determine if Repatterning and Brain Gym® activities would affect students' abilities to relax, coordinate, and cross the body midline in three dimensions. Ms. Stewart noted that the students' coordination improved, and some students' verbal, reading, eye-tracking, and social skills improved as well.

The Effects of Edu-K in a research project with second-graders. Al Milliren, Ed.D., ©1992. Published in *Brain Gym® Journal*, Volume X, No 2, 1996.
In 1992, South Carolina school counselor Dr. Al Milliren conducted a nonequivalent control group study investigating the impact of Brain Gym® on elementary school students who seemed to have potential yet who experienced difficulties in learning. After being tested for both auditory and visual perception, twelve second-grade students met in small groups twice weekly for five weeks. Each meeting included Brain Gym activities; some also included Edu-K balancing. Post-testing, conducted one month after the groups were concluded, indicated measurable improvements in visual skill performance but inconclusive changes in auditory skills. Dr. Milliren believes that results obtained from this small group might offer some direction for further exploration and research. His observations follow.

Using Brain Gym® for matching pitch. Donna Sewell, ©1992. Published in *Brain Gym® Journal* as "Field Study on Using Brain Gym for Matching Pitch, 1991-92." Volume VII, No 1 1993.
In 1991 and 1992, Donna Sewell, a Brain Gym® Instructor and elementary and secondary school teacher, conducted two pre-experimental pilot studies to investigate Brain Gym's efficacy in learning to match pitch. The studies were implemented at Taylorville High School in Salt Lake City, Utah, with the cooperation of the chorus teacher, Norm Wendel, and the permission of Dr. Ellis C. Worthen, Associate Director for Fine Arts for Granite School District in Salt Lake City. Participating students ranged in age from fifteen to seventeen years and had already been singing in a school setting for ten years.

Effect of Educational Kinesiology on hearing. Guruchiter Kaur Khalsa and Josie M. Sifft, ©1992. Published in *Brain Gym® Magazine*, Volume IV, No 3, ©1990.
This study was completed with 16 elementary school teachers who served as their own control. Each teacher was tested on the Pure-Tone audiometer before and after each movement experience. The movement experiences were 10 minutes of random movements about the room or a series of five Brain Gym activities. The results indicated that the hearing of the teachers was better after the Brain Gym activities than after the random movements.

The brain option for hyperactivity, ADD, E.H., Sp.Ed, L.D, and FAS. Carla L. Hannaford. ©1990. This essay hypothesizes that Brain Gym movements can eliminate or greatly ameliorate the symptoms of hyperactivity, learning disabilities, Attention Deficit Disorder, emotional handicaps, and even Fetal Alcohol Syndrome. A review of current brain literature supports the use of movement to enhance learning. (9 pages.) This research study is summarized in the Educational Kinesiology Foundation Research Packet as *The Effects of Brain Gym with Special Ed students grades three through five.* (See below)

The effects of Brain Gym with Special Ed. Students grades three though five. Carla Hannaford, M.A. ©1990.
In 1989-1990, Carla Hannaford, M.A., an educator and neurophysiologist, implemented a yearlong one group pre-test/post-test study in the Hawaii School District. Hannaford incorporated Brain Gym® in the classroom with nineteen fifth graders in Special Education. Pre- and post-tests were completed using the Brigance Inventory of Basic Skills. Post-tests showed a one-to-two-year growth for all students on the reading and comprehension testing and growth of one or more years for more than 50 percent of the students on math scores—greater results than might have been expected for Special Education students. Behavior patterns also improved.

Effects of Edu-K on computer-related eye and muscle strain. Joan Spalding, M.S., © 1990. Published in *Brain Gym® Magazine*, Volume V, No 2, 1991.
In 1990, Joan Spalding, M.S., of Mancato State University, conducted this one-group pre-test/post-test pilot study in partial fulfillment of the requirements toward her Master's Degree. The purpose of the study was to determine whether Edu-K Brain Gym® and Vision Gym activities have an effect on eye and muscle strain or other physical symptoms generated by use of a computer video display terminal (VDT). The project was conducted over a six-week period. Ten subjects from twenty-nine to fifty years who used the VDT as a principal part of their work (four or more hours daily) punctuated each hour of computer time with a five-minute break for Edu-K movements. Half the subjects were male, half female. Half were entrepreneurs, half salaried employees. Statistically significant results indicated that computer breaks for Edu-K activities contributed to a lessening of visual and muscle-related stress.

Effects of combined Brain Gym® and mountaineering experiences on teen and pre-teen scholastic achievement. George Gardner and Colleen Carroll-Gardner, ©1989.
In 1989, under the supervision of teachers George and Colleen Gardner, preteens and teenagers learned and implemented Brain Gym® for reading, communication, and mountaineering skills in a fourteen-day wilderness program in Colorado. In this one-group, pre- and post-test research design, results from each two-week program suggest that most participants initially performed as much as one grade level below their true potential. After learning and integrating the Brain Gym activities, participants were able to perform at an average level six to eight months higher than their baseline score.

The effects of Edu-K in a remedial summer school program. Helen Cox. ©1989. See also "Options in Health and Education: Developing Sensory Readiness," *Brain Gym® Journal*, Volume III, No 2, ©1989.
In 1989, Helen Cox, Director of the Options in Health and Education Learning Center, and Dr. Al Milliren implemented a one-group pre-test/post-test study measuring the effects of Educational Kinesiology and Char-L Intensive Phonics in a remedial summer school program held at Brimfield Public Grade School in Brimfield, Illinois. The program was funded by a Chapter One Federal

Grant. Post-test results on Slosson tests showed a greater increase in math and reading skills than could ordinarily be expected in a summer school program.

Effects of Edu-K on psychometric measures of achievements of Special Ed elementary students. Lark Carroll. ©1988.
In 1987-1988, Brain Gym Instructor Lark Carroll supervised this one-group, pre-test/post-test study in a Berkeley, California, public school Special Ed class. The study was funded with a $500 grant from Berkeley Public Education Foundation. The purpose of the study was to determine whether Repatterning and Brain Gym® used over an eight-month period would affect the word recognition, hand-eye coordination, and self-esteem skills of ten students in second and third grade. Results showed greater improvement on the standardized tests than would normally be expected.

Effects of Edu-K in the classroom on beginning reading skills. Dorothy H. L. Carroll, Ed.D. ©1987. Published in *Brain Gym® Magazine* as "Positive Activities," Volume II, No 2, 1988.
In 1987, twenty-two first graders in Pennsylvania, under the supervision of educator Dorothy H. L. Carroll, Ed.D., and classroom teacher Mary Ann Wittle, took part in this eight-week, one-group, pre-test/post-test study. The program consisted of Repatterning in the third and eighth week and about fifteen minutes a day of Brain Gym® activities. The purpose of this study was to determine whether the Edu-K techniques would affect students' recognition and reproduction of letters or numbers, their auditory and visual discrimination of sounds and words, or their ability to match and reproduce designs as assessed by standardized tests. The results indicated that nearly all of the children who made errors on the pre-tests improved their performances on those same tests ten weeks later.

A longitudinal perspective on Edu-K outcomes with Special Ed students in Australia.
Peter Whetton. ©1987.
In 1986-87, Peter Whetton, Senior Special Education instructor at Christies Beach High School in Christies Beach, Australia, implemented this informal project over three terms. The purpose of the project was to determine whether the inclusion of Brain Gym movements would have an effect on the behavior, coordination, attention span, or academic skills of high school students in a Special Education classroom. In Part One of the study, twelve students were divided into four groups: the Brain Gym group or one of three control groups. The results showed that the Brain Gym group improved markedly in all areas; the two control groups using movement showed small areas of improvement; the control group with no movement showed no improvement. In Part Two of the study, all students chose to do only Brain Gym for the nine-week period. Results showed continued improvements. In Part Three, Brain Gym was not used in the classroom for the eight-week period. Results showed that skills and behaviors declined until Brain Gym was reintroduced. A four-year longitudinal retrospective of participants is included, showing an average age growth of four to five years in reading, math, and spelling, and more than seven years' growth in comprehension.

Descriptive Research

Group I: Qualitative Research Designs

Results of a Brain Gym course at an educational program for underprivileged children in Bangladesh. Peter Winkelmann, © 2000.
In 1999, 10 UCEP (Underprivileged Children Educational Program) teachers completed their Brain Gym® training in Switzerland with instructor Perer Winkelmann. In February of the next year some of these trainees taught a three-day course for twenty school teachers in Drake, Bangladesh. Two months after the course, a questionaire was sent out to participants. Fifteen of the twenty questionaires were returned, yielding positive results in the areas of communication, behavior, academic performance, and health.

Brain Gym in a program for teachers and health staff, in North Sulawesi, Indonesia. Peter Winkelmann. © 2000.
Elisabeth Demuth, a trained nurse and certified Brain Gym instructor, works for a hospital-based primary healthcare project in the Province of North Sulawesi, Indonesia. Brain Gym courses have been incorporated into the training component of the project as part of its program for teachers and health staff in the province. Those courses are sold on a cost-recovering basis, with expenditure for overhead and promotion being covered by the Swiss funding agency. SOAM, as part of their regular support to this project. Mrs. Demuth has taught Brain Gym courses to more than 100 people in the province-mostly kindergarten and primary school teachers. This program is being positively received by the Implementing organization, GMIM Health Services, and by various governmental educational institutions. Questionaires sent to kindergarten teachers, six months after they had completed an introductory course, yielded the following information: All participants who responded to the survey used Brain Gym in class, either daily or two to three times per week; the following personal improvements were reported: an increase in joy at work, improved concentration, improved relations between teachers and children, improved working atmosphere; many participants reported that the performance of the children had increased, in particular: motor skills, learning by heart of songs and poems, concentration, speed in solving puzzles; all teachers would like to do an advanced course in Brain Gym.

Using Brain Gym with hearing impaired children in Flores, east Indonesia. Peter Winkelmann. © 1999. Beginning in 1997, I practiced Brain Gym (which we also refer to as Learning Gymestics) once a week with a group of twenty deaf children at the Handicapped Children's Home (Skolah Luar Blasa, SLB), Ruteng, Wastflores, for one and one half years. Besides noticing that the exercises were popular with the children, I observed that many of them made remarkable progress in several skills. When comparing the children's abilities in mathematics, reading, writing, and sports before and after practicing Brain Gym, measurable improvements were evident, which I have documented through a number of empirical surveys.

A year of Learning Gymnastics (Brain Gym) at Dorkes Kindergarten, North Sulewesi. Peter Winkelmann. © 1998. Dorkes Kindergarten in Tornohon has thirty children between the ages of five and six. It has three staff members, led by Mrs. Frida Ledi Lengkong. Her report of a year of regular Learning Gymnastics, consisting of fifteen-minute sessions in the morning and brief breaks during lessons is a follows: teaching and learning have become more animated and the concentration and participation of the children have improved; during the course of the year, the children learned by heart a record sixty songs and two plays; verbal expression has improved and

integrate the infant reflexes and brain dimensions through brain-integration technology such as the Brain Gym activities.

Correlates of Edu-K repatterning pre-checks with "at-risk" populations. Robert Eyestone
From 1987-1990,. Robert Eyestone, M.S., Educational Psychologist with the Weber County Mental Health Department, Weber, Utah, conducted three studies using the Edu-K Repatterning Pre-Checks to determine whether specific populations were using one-sided or cross lateral processing of visual and/or motor information. **In the 1987-88 study,** 257 of 270 participants tested from a population defined as "at risk" tested one-sided; 37 of 310 participants in the group not defined as at risk tested one-sided. **In the 1988-89 study,** 539 of 552 participants from at risk populations tested as one-sided processors. **In the 1989-90 study,** 202 of 204 participants from at risk populations tested as one-sided; 1 of 97 participants in the group not defined as at risk tested one-sided. This study was conducted specifically for the purpose of measuring the effectiveness of the screening device to determine ease of processing. Highly significant correlates were found between those tested as using homolateral processing and those in Resource, Handicapped or Juvenile Detention Centers. Results suggest that the Dennison test for laterality may be an effective tool for screening individuals for further testing.

Group III: Publications on Summary Research Designs

Impact of Brain Gym processes on sales of insurance. Robert Donovan. ©1993.
In 1993, the South Carolina Farm Bureau Insurance Company held an open enrollment for the Switched on Selling (SOS) seminar, and about one-third of the sales force elected to participate. Participants in the one-day course learned the Brain Gym® movements, experienced Dennison Laterality Repatterning, and explored the applications of these processes to specific aspects of the selling process. For 120 days following the seminar, the company tracked results for the SOS group as well as for those salespeople who did not attend the seminar. The results suggest that the SOS salespeople made a significant change in their performance. The SOS group increased the number of applications received for insurance policies by 39 percent, as compared to no increase for the control group. Similarly, the premiums earned by the SOS group went up 101 percent, as compared to only a 30 percent increase for the non-SOS group.

The results of preliminary and follow-up questionnaires administered to salespersons attending Switched-On Selling seminars. Jerry V. Teplitz, JD, Ph.D. ©1992.
This study, completed in 1992, analyzed the attitudinal changes of participants who attended a one-day Switched-On Selling seminar toward various elements of the sales process. Participants completed a questionnaire at the beginning of the class and again at the end of the session. The seminar did not teach technique; rather, it covered all aspects of the selling process, such as prospecting, presenting, and follow-up, and used preselected Brain Gym® activities. Eighteen questions were asked of each of the 149 participants. The two questions that revealed the highest level of change were "I handle rejection well" and "It is easy for me to make cold calls using the telephone." On the question "I handle rejection well," the number of salespersons in disagreement with this at the beginning of the seminar dropped from 56% to only 8% at the conclusion of the seminar. On the question "It is easy for me to make cold calls using the telephone," on the prequestionnaire only 42% agreed or strongly agreed with this question. At the end of the seminar, 90% responded "agree" or "strongly agree." The results indicated that Switched-On Selling could have a very positive impact on the attitudes of salespeople. What felt difficult at the beginning of the day felt easier to do at the end of the seminar.

readiness to ask questions has increased – a child who was to have been referred to a speech therapist improved so much that therapy became unnecessary; when the class moved on to primary school, their new teacher remarked on the increased vitality of Mrs. Lengkong's children.

Brain Gym for preschoolers in a Headstart program. Gail Dennison. ©1996.
In 1996 Gail Dennison, educator and co-author of the Brain Gym® program, and Diane Lehman, Brain Gym Instructor and nutritional consultant to Ventura County Headstart schools, implemented a five-week experimental Brain Gym program with fifteen preschoolers. The intent of the program was to support the development of readiness skills of posture and coordination and to help the children develop eye-teaming and listening skills for the near-point tasks of drawing, reading, and writing. Significant observational and anecdotal data were realized from the study. Results indicated many improvements for individual students as well as their teachers, suggesting areas for further study.

Effects of Brain Gym® in a district-wide Canadian field study. Nancy McGovern, P.T. ©1991.
In June of 1991, Nancy McGovern, District Physiotherapist for the Department of Special Services, implemented a qualitative small group study as a pilot program in School District twenty-four, Kamloops, British Columbia, Canada. The purpose of this study was to determine the possible inclusion of Brain Gym® movements in the curriculum for learning-disabled students. Originally, nine schools twelve teachers, fifteen key students and three "Sensory-Motor" P.E. groups were involved. Interest in this program eventually generated the involvement of one additional school, twenty-one teachers and twelve key students. Approximately 600 total students in ten schools were involved. Perceptual tests, parents feedback and teachers and students observations comprised the evaluation. Results indicated many improvements for individual students and for whole classes; suggested elements to consider further in terms of implementation were presented.

The effects of Edu-K on academic and social skills of high school students in Israel.
Jeanette Primost. ©1990. Published in *Brain Gym® Magazine*, Volume VI, No 1, 1992.
In 1989 and 1990, Jeanette Primost used Edu-K with twelve high school students in Tel-Chai, Israel. She saw each pupil weekly for six or more sessions. The first session began with a Wonder Balance for eyes, ears, writing, and whole-body movement. She let the students direct her as to what they needed, using the Edu-K balance format from both basic and In-Depth processes. This qualitative, small-group report was coauthored by Jeanette and Chana Shar'abi, head of the learning center, who gathered data on students' learning skills, motivations, and personal feelings from exams, teachers, parents, and students themselves. Results indicated that seven pupils out of twelve benefited noticeably.

Group II: Publications on Correlational Research Designs

The influence of Brain Gym® movements on the work of muscles and on dynamics and posture reflexes. Svetlana K. Masgutova, Ph.D., © 1999. See also "Edu-K and Vigotsky's Mind-Body Psychology," *Brain Gym® Journal*, Volume X, No 3, 1996; "Educational Kinesiology in Russia: The Possibilities in Education and in Psychological Practice," Volume IX, No 3, 1996; and "Brain Gym® in Russia: Applications in Psychological Practice."
This descriptive study was designed to identify the correlation between infant reflexes, specific muscle groups, specific Brain Gym activities, and the Three Dimensions of learning, as identified by Paul and Gail Dennison. The high correlation identified in a population of 522 children "at risk" suggests the need for educators to address the physical development of children, enabling them to