#1345

AIDS VACCINE

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Joan Claybrook, President

Jerome P. Kassirer, MD
Editor-in-Chief
Marcia Angell, MD
Executive Editor
New England Journal of Medicine
10 Shattuck Street
Boston, MA 02115-6094

August 8, 1994

Dear Drs. Kassirer and Angell:

We have just come into possession of documents not previously made public that provide conclusive evidence of statistical manipulation and selective data presentation by Lt. Col. Robert Redfield, MD, and his colleagues. Dr. Redfield was then Chief of the Department of Retroviral Research at the Walter Reed Army Institute of Research (WRAIR) and is now with the Walter Reed Army Medical Center in Washington, DC. This misconduct occurred in the lead article in the *New England Journal of Medicine* (NEJM) on June 13, 1991 (see Attachment 1) as well as a letter published in the Journal on January 30, 1992 (see Attachment 2), reporting the results of trials of the GP160 AIDS vaccine (VaxSyn; MicroGeneSys, Meriden, Connecticut), which is intended to prevent disease progression in persons infected with HIV. We call on you to immediately demand a formal retraction of these misleading publications by Dr. Redfield and his co-authors.

Figure 4 from NEJM Article employing "smoothed" data

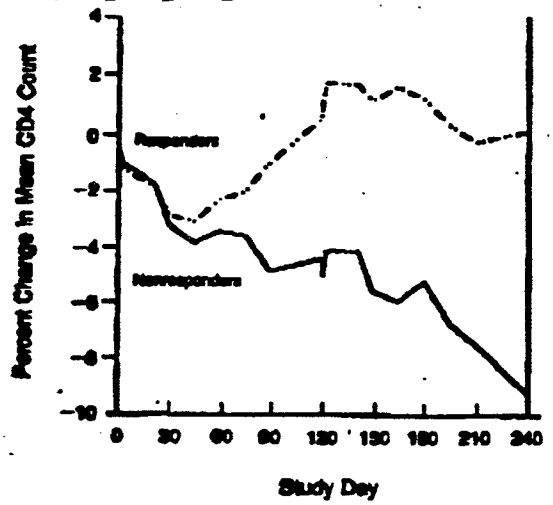
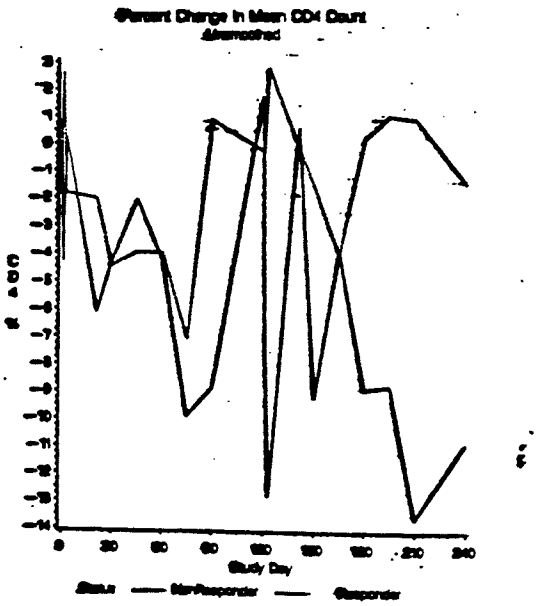


Figure 4. Change from Base Line in the CD4 Cell Count in Responders and Nonresponders.

Each base-line value represents the mean CD4 count at all pre-immunization evaluations performed during the two months before the initial vaccination (four to six values per subject). Each point represents a moving average of seven values

Actual raw data from study



In Figure 4 of the NEJM article (see above, left) and the accompanying text, Dr. Redfield and colleagues present data purportedly demonstrating that vaccine responders (those developing antibodies to the vaccine) had essentially stable CD4 cell counts (an index of immune function in persons infected with HIV), while the counts of non-responders declined. However, this striking visual effect is in large part the product of statistical manipulation by the authors. To produce Figure 4, the authors used a statistical technique called the moving average (in this case a moving average of seven sequential data points) to reduce the actual fluctuations in the subjects' CD4 cell counts (smoothing). After the Amsterdam AIDS Conference, where a similarly misleading presentation of these data was made, Col. Donald Burke, MD, Director of the Division of Retrovirology at WRAIR and then Dr. Redfield's immediate supervisor, requested that Dr. William McCarthy and his staff analyze the same raw data set without any smoothing techniques (see above, right).[1]

It is obvious that the use of the smoothing technique has produced an apparent benefit for vaccine responders, while the unsmoothed data provide no evidence of this. Rather, the raw data portray a series of seemingly random CD4 cell count variations in both responders and non-responders. In Figure 4, after the first 30 days, the CD4 counts of responders are consistently higher than those of non-responders throughout the rest of the 240 days. In the raw data, however, at four times after the first 30 days, CD4 counts for non-responders are actually as high or higher than the CD4 counts of responders. Indeed, statistical analysis by Dr. McCarthy confirms that there is no statistically significant difference in rates of CD4 cell count decline between responders and non-responders (see Attachment 3). The textual accompaniment to this misleading data presentation in Figure 4 was similarly misleading in stating that "the rate of decline in the CD4 cell count---was favorably influenced among the subjects, especially those classified as responders..."

Moreover, in an apparent attempt to demonstrate vaccine efficacy in stabilizing CD4 cell counts, Figure 4 was selected from a family of different types of moving average or "smoothed" plots generated from the same data set (see Attachment 4). Dr. Redfield asked his statisticians to produce graphs that used different moving averages and had different vertical axes. From the six graphs so generated, Dr. Redfield selected graph A, the graph demonstrating the greatest apparent benefit, to serve as Figure 4 in the paper published in the Journal. In a telephone conversation with one of us (PL) on June 22, 1994, Col. Donald Burke, MD, agreed that there were problems with Figure 4 and that the version published "was the one that looked the best."

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[1] At the time Dr. McCarthy was Director of Biostatistics for the Henry M. Jackson Foundation, a non-profit foundation created by an act of the U.S. Congress to work with Department of Defense researchers.

We have two additional concerns about the paper. First, the authors use the Discussion section of the Journal article to present an entirely new analysis: a comparison between subjects in the trial and those in a group of historical controls who did not receive the vaccine (see Attachment 1, p. 1683). The authors report an 8.7% decline in CD4 cell count among controls compared to a decline of 7.2% and 0.6% in those receiving the vaccine under two different dosing schedules. In the Journal letter, the authors present 24-month follow-up data on what are apparently the same subjects (see Attachment 2). The authors report that the CD4 cell counts declined 8.5% among vaccine recipients compared to 26.1% among the historical controls. We believe that it is inappropriate for the Journal to permit the use of the Discussion section of articles and its Letters section to present new analyses in a manner so sketchy that it prevents the reader from adequately assessing the data.

Second, in the Journal article the authors use the Toxicity section of their report to describe the amount of HIV in the subjects' blood (see Attachment 1, p. 1682). Without presenting any data, the authors state that "Assay by quantitative culture and the polymerase chain reaction demonstrated no changes during this trial." To permit such assertions to be published without any supporting data seems to us extremely inappropriate.

As you know, as a result of our June 7, 1994 letter to Representative Henry Waxman, the Congressman has agreed to conduct an investigation into these and other allegations of scientific misconduct by Dr. Redfield and his colleagues. One of you (MA) commented on the allegations detailed in our letter by saying: "As long as the data are reliable, and nobody disputes the raw data, people can choose to analyze it many different ways .... The analysis and the interpretation is to some extent a matter of opinion" (*Hartford Courant*, June 30, 1994). We believe that the documents presented here prove that analyses such as the one in Figure 4 of the Journal article stretch and smooth the raw data beyond recognition. We hope for a quick response to this urgent matter which we know is of concern to you. It is hoped that by now seeing the raw data upon which one of the major distortions---Figure 4 in the NEJM paper---was based, you will be helped in your decision.

Sincerely,

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ATTACHMENT 1

The New England
Journal of Medicine

Volume 324 JUNE 13, 1991 Number 24

A PHASE I EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF VACCINATION WITH RECOMBINANT gp160 IN PATIENTS WITH EARLY HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Abstract *Background.* Despite multiple antiviral humoral and cellular immune responses, infection with the human immunodeficiency virus (HIV) results in a progressively debilitating disease. We hypothesized that a more effective immune response could be generated by post-infection vaccination with HIV-specific antigens.

Methods. We performed a phase I trial of the safety and immunogenicity of a vaccine prepared from molecularly cloned envelope protein, gp160, in 30 volunteer subjects with HIV infection in Walter Reed stage 1 or 2. The vaccine was administered either on days 0, 30, and 120 or on days 0, 30, 60, 120, 150, and 180. HIV-specific humoral and cellular immune responses were measured; local and systemic reactions to vaccination, including general measures of immune function, were monitored.

Results. In 19 of the 30 subjects both humoral and cellular immunity to HIV envelope proteins increased in response to vaccination with gp160. Seroconversion to selected envelope epitopes was observed, as were new T-cell proliferative responses to gp160. The response was associated with the CD4 cell count determined before vaccination (13 of 16 subjects [81 percent] with >600 cells per milliliter responded, as compared with 6 of 14 [43 percent] with ≤600 cells per milliliter; P = 0.07) and with the number of injections administered (87 percent of subjects randomly assigned to receive six injections responded, as compared with 40 percent of those assigned to three injections; P = 0.02). Local reactions at the site of injection were mild. There were no adverse systemic reactions, including diminution of general in vitro or in vivo cellular immune function. After 10 months of follow-up, the mean CD4 count had not decreased in the 19 subjects who responded, but it had decreased by 7.3 percent in the 11 who did not respond.

Conclusions. This gp160 vaccine is safe and immunogenic in volunteer patients with early HIV infection. Although it is too early to know whether this approach will be clinically useful, further scientific and therapeutic evaluation of HIV-specific vaccine therapy is warranted. Similar vaccines may prove to be effective for other chronic infections. (N Engl J Med 1991; 324: 1677-84.)

INFECTION with human immunodeficiency virus type 1 (HIV) causes chronic progressive immunologic dysfunction.[1,2] Although the precise mechanisms of HIV-induced immune defects remain to be elucidated, the development of immunologic dysfunction as a result of HIV infection is well documented.[1-8] Longitudinal studies of HIV-infected cohorts have demonstrated a predictable rate of decline in the CD4 cell count and a relation between the CD4 cell count and survival.[3,4,9-12] Accordingly, HIV infection can be clinically classified into distinct prognostic stages on the basis of increasing degrees of immunologic dysfunction.[13]

Immune responses to HIV antigens are elicited during natural infection, and these may be important in regulating viral replication. Both humoral mechanisms (i.e., neutralization antibody, viral-receptor–blocking antibody, and antibody-dependent cytotoxicity) and cellular mechanisms (i.e., natural-killer-cell activity, HIV antigen–specific T-cell proliferative responses, and cytotoxic T-cell responses) have been reported.[14-26] Yet, despite these immune responses, HIV infection results in a progressive, debilitating disease of the immune system. The burden of HIV in vivo has been shown to increase in the later stages of infection[27-29]; some investigators have deduced that this is a consequence of viral-directed events such as changes

From the Department of Retroviral Research (R.R.R., D.L.B., V.P.) and the Division of Retrovirology (D.S.B.), Walter Reed Army Institute of Research (E.T.), Rockville, Md.; the Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, D.C. (J.F.B.); the Infectious Disease Service, Department of Medicine, Walter Reed Army Medical Center, Washington, D.C. (C.D., S.J., C.O.); the Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, Md. (N.K., T.W.); SRA Technologies, Inc., Rockville, Md. (A.F.); the Department of Biochemistry, Israel Institute of Biological Research, Ness-Ziona, Israel (A.S.); and MicroGeneSys, Inc., Meriden, Conn. (G.S., F.V.). Address reprint requests to Dr. Redfield at the Department of Retroviral Research, Walter Reed Army Institute of Research, 13 Taft Ct., Rockville, MD 20850.

Table 3. Immune Responsiveness to Vaccination, According to Immunization Schedule and Base-Line CD4 Count.

Schedule and CD4 Count	All Subjects	Responders	Nonresponders
	no.	no. (percent)	
Schedule A			
>600	7	5 (71)	2 (29)
500–600	5	1 (20)	4 (80)
<500	3	0	3 (100)
Subtotal	15	6 (40)	9 (60)
Schedule B			
>600	9	8 (89)	1 (11)
500–600	2	2 (100)	0
<500	4	3 (75)	1 (25)
Subtotal	15	13 (87)	2 (13)
Total	30	19 (63)	11 (37)

decline in the CD4 count of any subject throughout the entire course of the trial.

To assess the possibility of increased HIV replication and viral load in the subjects as a consequence of vaccination, in vivo viral activity was measured by quantitative cultures of the virus in plasma and peripheral-blood mononuclear cells, by the polymerase-chain-reaction testing of DNA from peripheral-blood mononuclear cells, and as serum levels of p24 antigen. Assay by quantitative culture and the polymerase chain reaction demonstrated no changes during this trial. Serum p24 antigen was undetectable in all subjects.

Discussion

The therapeutic use of vaccines was introduced by Pasteur in the 19th century for the treatment of acute rabies infection, but the value of this approach in the treatment of other infections has not been extensively explored. Although there are other examples of postinfection modification of viral-specific immunity (for example, after exposure to hepatitis A or B), there are no well-documented studies in humans that have demonstrated the feasibility of this approach in the setting of an established or chronic viral infection. Even in animals the only suggestion that such an approach is feasible is limited to a single investigation of herpes simplex in guinea pigs.[48]

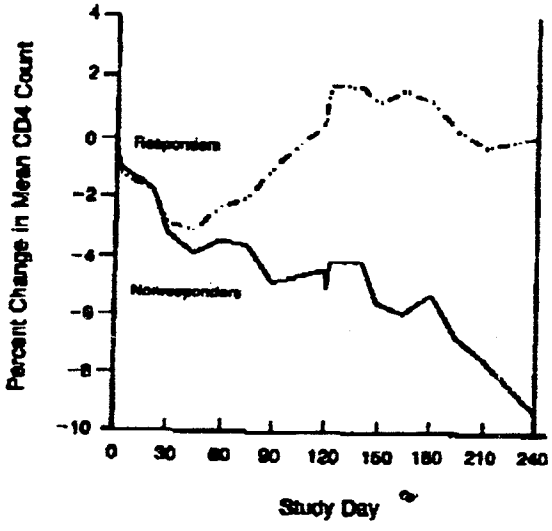

Figure 4. Change from Base Line in the CD4 Cell Count in Responders and Nonresponders.

Each base-line value represents the mean CD4 count at all pre-immunization evaluations performed during the two months before the initial vaccination (four to six values per subject). Each point represents a moving average of seven values (see Methods).

The present study demonstrates the feasibility of virus-specific immune modification by active immunization after infection. Specifically, a gp160 vaccine derived from an HIV envelope gene augmented host-directed viral-specific humoral and cellular responses in 19 of 30 persons with early HIV infection. The definition of vaccination response that we chose — i.e., the requirement that a response be both humoral and cellular — was arbitrary but highly restrictive in the light of the scientific objective of this trial to assess the feasibility of postinfection immunization, and in the absence of support for this concept in studies of other chronic viral infections.

By qualitative and quantitative measurement of distinct antibody responses to specific HIV epitopes in natural infection as opposed to postinfection immunization, vaccine-induced humoral immunogenicity in already infected persons was documented in 70 percent of the subjects. Although gross analysis of whole viral proteins by the Western blotting technique was helpful, characterization of humoral response by mapping of distinct epitopes proved to be a more sensitive method of assessing immunogenicity. Seroconversion to specific envelope epitopes occurred in 20 subjects (19 vaccine responders and 1 nonresponder) (Table 2). In addition, seroconversion associated only with vaccination (conversion to epitopes 241, 254, and 342) occurred in 10 subjects. This variation in humoral responses to the gp160 vaccine, as characterized by epitope mapping, will permit prospective cause-and-effect analysis of specific antibody responses and presents unique opportunities to characterize potential immunoregulatory mechanisms not elicited during a natural infection.

Although the relevance of serum neutralizing activity in vivo is unknown at present, the observation of increased neutralizing activity against disparate strains of HIV (IIIB, RF, and MN) in four of five responders suggests that postinfection immunization induced changes in functional antibody. This vaccine-induced increase in serum neutralization capacity against distinct strains of HIV will potentially aid in the definition of group-specific neutralization epitopes.

A proliferative response to HIV envelope proteins rarely occurs in natural HIV infection (data not shown). After immunization with gp160, however, specific T-cell proliferative responses were documented in 21 (70 percent) of the subjects. The reason for this difference is unclear. One possibility is that the new proliferative response may be directed against an envelope epitope (or epitopes) unique to the vaccine

(as a result of the methods of vaccine production or antigen processing in vivo). Alternatively, the protein used in the proliferation assay may not stimulate primary T-cell proliferative responses against homologous wild-type envelopes of natural virus. We have recently obtained additional evidence that vaccination may boost the host cellular immune response: in selected responders to vaccination, HIV-IIIB type–specific cytotoxic T-cell responses were induced after booster immunization (data not shown).

The factors responsible for immunoresponsiveness to vaccination in HIV-infected persons remain to be clarified. Even in early HIV infection, individual patients respond suboptimally to a variety of vaccines, as compared with matched controls.[49] This hyporesponsiveness has been related to early B-cell dysregulation and T-cell dysfunction.[31,50] In the present trial, immunoresponsiveness to vaccination was associated with the base-line CD4 cell count, a finding consistent with the hypothesis that the immunologic status of a host is an important determinant of responsiveness. However, the immunization schedule within specific T-cell–count intervals (Table 3) also influenced responsiveness: schedule B (six injections) was superior. Indeed, the decreased response observed in the subjects with lower CD4 cell counts could be improved by an increased number of vaccinations, which suggests that further modifications in the dosage, regimen, adjuvant treatments, or formulation may improve host immunoresponsiveness.

Although questions have been raised about the safety of active immunization of HIV-infected persons with HIV-specific vaccine products,[51] there was no evidence of immune-specific toxicity. Quantitative cultures, DNA polymerase-chain-reaction assays, and serum antigen assays did not document any evidence of increased HIV load in vivo. Moreover, an excellent in vivo surrogate marker of HIV replication — the rate of decline in the CD4 cell count — was favorably influenced among the subjects, especially those classified as responders, in whom the decrease in the mean CD4 count was 0.2 percent, as compared with 7.3 percent in nonresponders. These data demonstrate that postinfection immune responsiveness was not associated with an increase in CD4 cell destruction, but perhaps rather with decreased replication of HIV in vivo. A more direct measurement of in vivo active expression of virus — RNA-transcript analysis — is under development.[52]

An open, unblinded, phase I trial is not designed to provide conclusive information about therapeutic efficacy. Thus, the ability to respond to gp160 with either a primary or a secondary immune response may have been restricted to a subgroup of patients who had less severe B-cell or T-cell dysfunction. The difference observed between the base-line mean CD4 counts of responders and those of nonresponders (716 and 605 cells per milliliter, respectively) and the overall poor response of subjects with CD4 counts of 600 cells or fewer per milliliter at entry support this possibility. However, because of the grim prognosis of patients with this infection, we believed it was important to explore potential clinical benefits. Thus, we retrospectively compared changes in the subjects' mean CD4 cell counts according to treatment group (vaccination schedules) with expected changes observed during untreated infections, using a data base on the natural history of HIV infection in a cohort of patients from the U.S. Army. Ten patients from this cohort were matched for age, ethnic group, and base-line CD4 cell count with each subject. The mean CD4 count decreased by 8.7 percent in this historical reference group, decreased by 7.2 percent in subjects assigned to schedule A, and increased by 0.6 percent in subjects assigned to schedule B. Although preliminary, these results are encouraging. Direct evidence of therapeutic benefit must await the completion of phase II studies of clinical efficacy.

In the light of these results, the scientific and therapeutic importance of HIV-specific immunization warrants further investigation. Postinfection vaccination should serve as a powerful tool to further the understanding of HIV immunoregulation and, if proved clinically relevant, would provide an alternative strategy for treatment. This approach may also prove useful in defining a protective immune response (or responses) relevant to the prophylactic use of vaccines.

We are indebted first and foremost to each of the trial subjects for their dedication, cooperation, and courage; to the technical staff of the Department of Retroviral Research, the Henry M. Jackson Foundation, and SRA Technologies for their contributions, especially Sonya Dilworth, Kathryn Kersey, Cheryl Lewis, Kathleen Tencer, and Maria Wood; to the technical and administrative staff of MicroGeneSys for their efforts, especially Alex Toles, Carol Smith, and Michael Smith; to the administrative staff of the Henry M. Jackson Foundation, especially John Lowe, Mary Hall, Victoria Hunter, Dr. Lou Lorton, Sherry White, and Joan Loveland, for their support; to the protocol nurses of the Henry M. Jackson Foundation, especially Linda Bean, Paul Kernozek, and Mercy Swatson, for their meticulous clinical execution of this trial; to the pharmacy service of Walter Reed Army Medical Center, especially LTC Darrel Bjornson, for their support; to the clinical staff of the Walter Reed Army Medical Center for their clinical care of the patient volunteers; to Dr. Philip K. Russell and Dr. Jay P. Sanford for their review of the manuscript and their helpful comments; and to Dr. Michael Scotti for his encouragement and support.

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ATTACHMENT 2

tients infected with herpes with HSV vaccines might enhance host immune responses and result in improved control of recurrent disease.[1]

Initial attempts to test this hypothesis were hampered by poor study design and weakly immunogenic vaccines.[2] Recently, however, studies in laboratory animals that have used either vaccines derived from cell culture or recombinant HSV glycoprotein vaccines have shown that both symptomatic recurrences and subclinical HSV shedding from the genital tract can be reduced when the vaccines are administered after the establishment of persistent infection.[3-5] In the guinea pig, the administration of vaccine after infection stimulates virus-specific cell-mediated immune responses that may be critical to the control of recurrent herpetic infections.[6] Efficacy required the simultaneous administration of potent adjuvants.[6] In that system, aluminum phosphate (the adjuvant used by Redfield et al.) enhanced humoral immune responses but was not effective in reducing recurrent HSV disease.[7]

Although immunotherapy using glycoprotein vaccine for persistent genital HSV infections in guinea pigs may not be directly analogous to immunotherapy with glycoproteins for chronic or persistent HIV infections in humans, we suggest that these data and the promising preliminary studies by Redfield and colleagues warrant proceeding with trials to determine whether clinical improvement can be produced by immunizing HIV-infected patients with vaccines derived from HIV subunits.

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The authors reply:

To the Editor: Dr. Oyaizu and colleagues raise questions about the safety of HIV-envelope–based vaccines. However, the extrapolation of in vitro events to in vivo consequences requires experimental verification. Because a major objective of a phase I trial is to assess safety, we staggered our enrollment, beginning with only two volunteers. In 1989 the safety of HIV-envelope–based vaccine therapy in the setting of chronic HIV infection was scientifically unknown; the courage of these volunteers in the original trial should not be underestimated. The results of our trial document the short-term (eight months) in vivo safety of vaccinating HIV-infected volunteers with baculovirus-derived gp160. Safety was demonstrated both clinically and immunologically by quantitative CD4 counts, and qualitative T-cell function was shown in vitro by mitogen- and antigen-specific proliferation and in vivo by the assessment of delayed-type hypersensitivity skin-testing antigens.

CD4-cell destruction is a direct consequence of HIV–host interaction, and the rate of CD4 decline in an HIV-infected population is predictable. The HIV-infected volunteers vaccinated with the six-injection regimen had stabilization of their CD4 counts, with a decrease in the count of 3.3 percent, as compared with a decline of 8.7 percent in the matched natural-history cohort. A difference in CD4 decline persisted through 24 months of follow-up, at which time the decline was 8.5 percent in the volunteers and 26.1 percent in the matched natural-history group. Throughout the trial, antigen-induced proliferation and skin-test responses remained undiminished. Although these data do not prove clinical efficacy, they do support the safety of this vaccine in persons with early HIV infection.

We disagree with the closing comments of Dr. Oyaizu and colleagues. Many chronic viral diseases result in disease after the development of a host-directed specific antiviral immune response (i.e., herpes simplex, varicella, cytomegalovirus, hepatitis B virus, and human T-cell lymphotropic virus Type I). The immunoregulatory mechanisms responsible for effective postinfection control of HIV are unknown. In natural HIV infection, host-directed immune responses against gp120 are minimal. They include epitope-specific antibody response to constant regions 1, 2, and 3; T-cell recognition and proliferation; and cytotoxic T-lymphocyte responses. These anti-envelope responses are generated after vaccination even though the host failed to develop them as a consequence of natural infection. The fact is that natural infection with HIV does not define the limits of a person's immune response directed against gp120; the ability to broaden anti-envelope immune responses underscores the potential of this exciting treatment strategy.

As noted by Dr. Seligman, we observed that immunization with gp160 altered the volunteers' capacity to neutralize HIV-IIIB (the vaccine strain) and the MN and RF strains. Alterations in neutralization responses to each prototype did not correlate with vaccine-induced binding antibody to the V3 loop of IIIB and MN, although this does not exclude the possibility of a correlation with conformationally dependent interactions. In an attempt to clarify the complex issue of group neutralization and genomic diversity, we have developed a neutralization assay for peripheral-blood mononuclear cells that uses paired viral isolates and serum samples from patients; it is currently being used with our phase I patients.

Finally, we appreciate the work and comments of Dr. Stanberry and his colleagues. We believe that vaccination after infection offers a new tool to expand our scientific understanding of virus–host interaction and the immunoregulation of chronic human pathogens. It is most important to explore this new therapeutic strategy and to assess its ultimate clinical potential in the treatment of chronic viral disease.

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FETAL PROTECTION AND JOB DISCRIMINATION

To the Editor: With regard to the Johnson Controls case, discussed by Annas in the *Journal* (Sept. 5 issue),[1] although the decision reached was "consistent with good medical practice,"[1] the analysis employed in reaching that decision is problematic.

We are told, "*Johnson Controls* holds that recasting sex discrimination in the name of fetal protection is illegal."[1] Unfortunately, the majority opinion in *Johnson Controls* goes further. Determining that protecting the safety of fetuses (or other noncustomer third parties) is not reasonably related to either the "essence of the [battery-making] business" or the "essence of the job," the Court says that sex-specific third-party–protection policies are indefensible under Title VII.

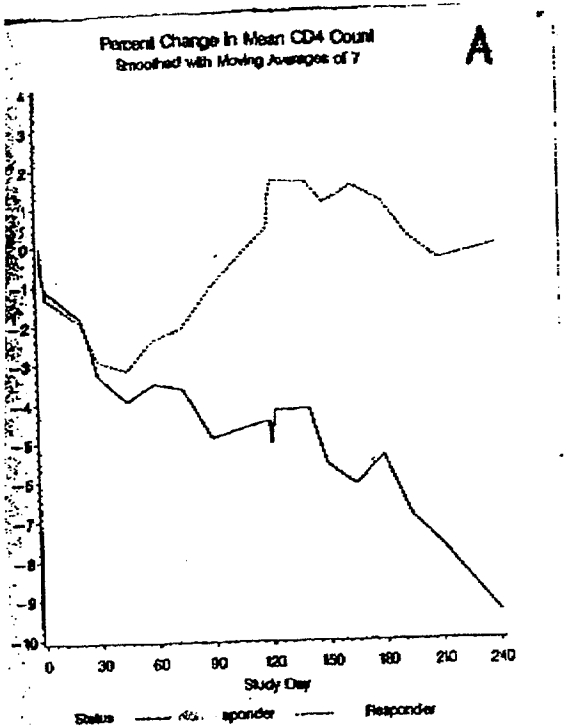
This is unfortunate. Under "the Court's narrow interpretation of the [bona fide occupational qualification, or BFOQ] defense . . . an employer cannot exclude even *pregnant* women from an environment highly toxic to their fetuses."[2] For example, under the Court's restrictive view of the BFOQ defense, a U.S. producer of blended Scotch whiskey could not exclude a pregnant woman from being employed as a taster, despite the relation between even low levels of

Re-Analysis of NEJM Fig. 4. [responders vs. non-responders; as defined by RRR in the NEJM article].

Used actual raw patient CD4 data that was used in the NEJM Fig. 4 Statistical methods used outlined in McCarthy Army statement and earlier McCarthy memos.

	t-test	Wilcoxon
Slope (uses all data points)	p=0.13	p=0.14

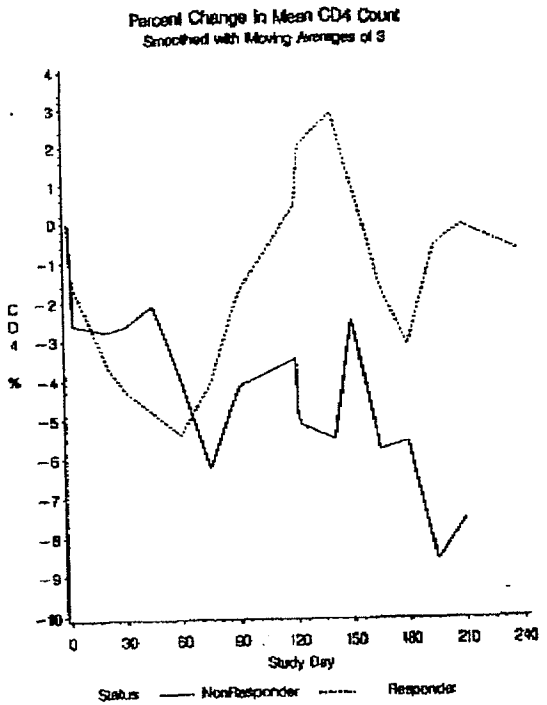
A


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 7
Study Day
Status — NonResponder — Responder

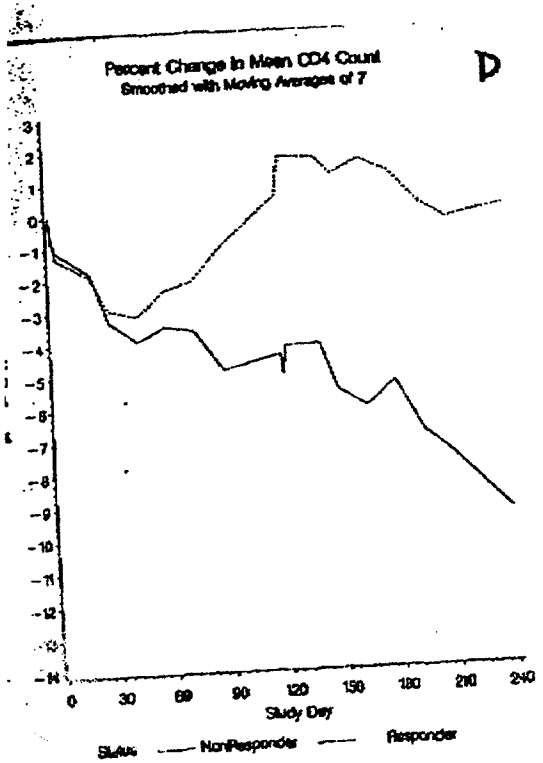
B


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 5
CD4 %
Study Day
Status — NonResponder — Responder

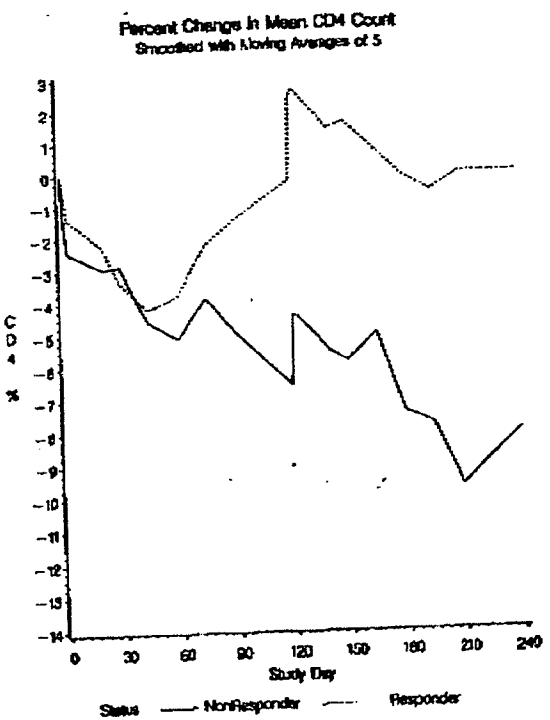
C


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 3
CD4 %
Study Day
Status — NonResponder — Responder

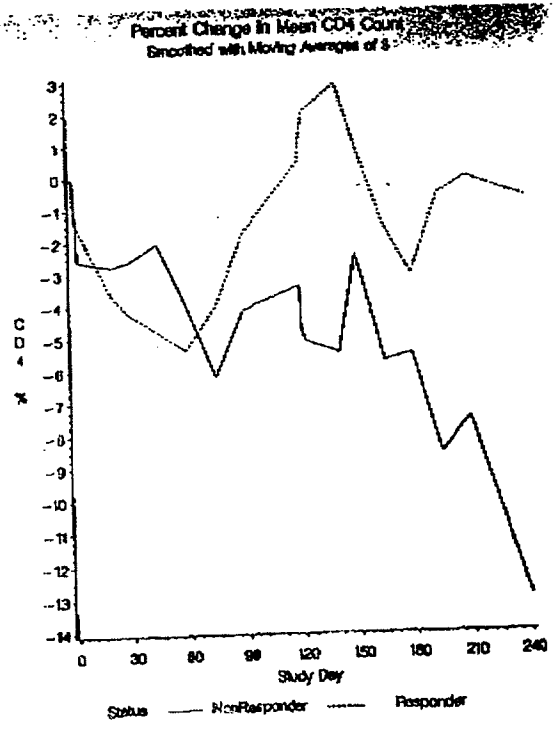
D


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 7
Study Day
Status — NonResponder — Responder

E


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 5
CD4 %
Study Day
Status — NonResponder — Responder

F


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 3
CD4 %
Study Day
Status — NonResponder — Responder

Public Citizen

Buyers Up • Congress Watch • Critical Mass • Health Research Group • Litigation Group

Joan Claybrook, President

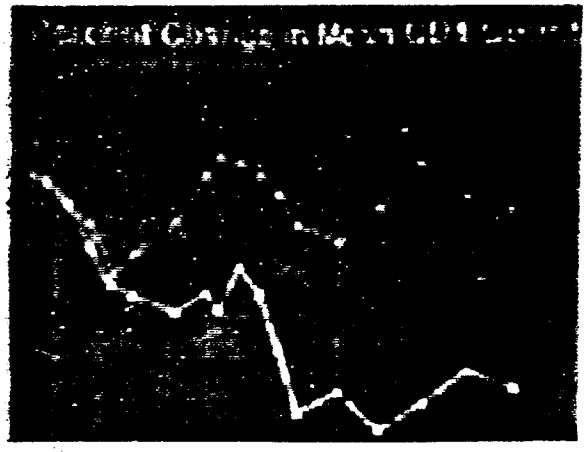
Dr. Peter Piot
International AIDS Society
c/o Institute of Tropical Medicine
Nationalestraat 155 B-2000
Antwerp, Belgium

August 8, 1994

Dear Dr. Piot:

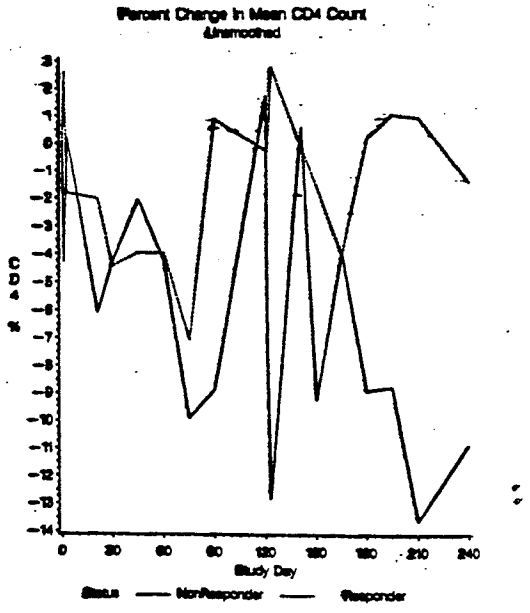
We are writing to urge the International AIDS Society to 1/ demand that Lt. Col. Robert Redfield, MD and his co-authors retract, in an oral presentation as well as in writing, a presentation on the GP160 therapeutic AIDS vaccine at the 1992 International AIDS Conference in Amsterdam, the Netherlands; and 2/ censure Dr. Redfield and his co-authors for selective and misleading data presentation and inappropriate statistical analyses in that presentation. Dr. Redfield was then Chief of the Department of Retroviral Research at the Walter Reed Army Institute of Research (WRAIR) and is now with the Walter Reed Army Medical Center in Washington, DC. Dr. Redfield's July 21, 1992 presentation at the conference addressed 1/ changes in the CD4 cell counts (an index of immune function in persons infected with HIV) of vaccinees; and 2/ changes in the viral load (the amount of HIV in a subject's blood) among vaccinees compared to a historical control group that did not receive the vaccine.

Amsterdam: Smoothed CD4 counts



See Attachment 4 for a clearer, published version.

Actual raw data from study



1. CD4 Cell Counts

The slide depicting CD4 cell count trends presented at the 1992 Amsterdam AIDS conference by Dr. Redfield (above, left) was similar to Figure 4 in an article published by Dr. Redfield and his colleagues in the June 13, 1991 issue of the *New England Journal of Medicine*. We have recently come into possession of documents not previously made public that clearly establish that the misuse of statistical techniques in large part obscures the actual findings and explains the stabilization of CD4 cell counts claimed by Dr. Redfield.

To produce Figure 4 and the similar slide presented in Amsterdam, the authors used a statistical technique called the moving average (a moving average of several sequential data points) to reduce the actual fluctuations in the subjects' CD4 cell counts (smoothing). Following the Amsterdam presentation, at the request of Col. Donald Burke, MD, Director of the Division of Retrovirology at WRAIR and then Dr. Redfield's immediate supervisor, Dr. William McCarthy and his staff analyzed the same raw data set without any smoothing techniques (see above, right for raw data).[1]

It is obvious that the use of the smoothing technique (above, left) has produced an apparent benefit for vaccine responders, while the unsmoothed data provide no evidence of this. Rather, the raw data (above, right) portrays a series of seemingly random CD4 cell count variations in both responders and non-responders. In the smoothed data, after the first part of the experiments, the CD4 counts of responders are consistently higher than those of non-responders throughout the rest of the 360 days. In the raw data, however, at four times after the first 30 days, CD4 counts for non-responders are actually as high or higher than the CD4 counts of responders. Indeed, statistical analysis by Dr. McCarthy confirms that there is no statistically significant difference in rates of CD4 cell count decline between responders and non-responders (see Attachment 1). Dr. Redfield was quoted in both the *New York Times* of June 26, 1992 and the *Wall Street Journal* of July 15, 1992 as saying that there was stabilization of CD4 cell counts among vaccine recipients.

Moreover, in an apparent attempt to demonstrate vaccine efficacy, Figure 4 was selected from a family of plots generated from the same data set (see Attachment 2). Prior to the submission of the *New England Journal of Medicine* paper, Dr. Redfield asked his statisticians to produce graphs that used different moving averages and had different vertical axes. From the six graphs so generated, Dr. Redfield selected graph A, the

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[1] At the time Dr. McCarthy was Director of Biostatistics for the Henry M. Jackson Foundation, a non-profit foundation created by an act of the U.S. Congress to work with Department of Defense researchers.

graph demonstrating the greatest apparent benefit, to serve as Figure 4 in the paper published in the *New England Journal of Medicine*. In a telephone conversation with one of us (PL) on June 22, 1994, Dr. Burke agreed that there were problems with Figure 4 and that the version published "was the one that looked the best." Although the data presented at the Amsterdam AIDS conference does not exactly match the family of "smoothed" curves, it is much more similar to them than to the actual raw data. The Amsterdam slide covered 360 days, Figure 4 in the New England Journal, 240 days.

2. Viral Load

In his oral Amsterdam presentation, Dr. Redfield also presented slides (see Attachment 3) purportedly demonstrating statistically significant decreases in viral load among vaccinees compared to a historical control group. In a presentation at the conference, later aired on CBS-TV in the U.S., Dr. Redfield described the reported decrease in viral load: "The virus [load] goes down. These are quite strong, significant, real, reproducible observations." However, although he had been given data for all 26 patients with viral load analyses prior to the conference, the data presented for the vaccinated patients were for only seven subjects in one slide and 15 subjects in another.

In August 1992, after questions about the Amsterdam presentation were raised, Dr. McCarthy and Lt. Col. John Brundage, head of Epidemiology at WRAIR, were called in to separately analyze the raw data but were unable to replicate Dr. Redfield's results for the viral load. Dr. Redfield agreed at an internal Department of Defense meeting on August 28, 1992 that his Amsterdam statements regarding viral load had been incorrect, that the control group used had been inappropriate and that the full data set should be used in the analysis. He made similar admissions on at least two subsequent occasions to internal Department of Defense audiences.

Two years have now elapsed since these incorrect and misleading analyses were presented in Amsterdam and widely covered by the popular press. It is also almost two years since Dr. Redfield admitted in internal Department of Defense meetings that his findings were faulty, yet no formal retraction of the data has ever been made. In addition to censuring Dr. Redfield and his co-authors for this egregious scientific misconduct, we ask that they be required at this or the next International meeting to formally retract the data in an oral presentation of equal prominence to the original Amsterdam presentation and that they be required to print a formal retraction in the abstract book for the next International AIDS Conference.

We are also writing today to the *New England Journal of Medicine* requesting a retraction of the June 13, 1991 article as well as a related letter by Dr. Redfield and his colleagues published on January 30, 1992.

Sincerely,

Peter Lurie, MD, MPH
Assistant Adjunct Professor
University of California
San Francisco
Research Associate
Public Citizen's Health Research Group

Sidney M. Wolfe, MD
Director
Public Citizen's Health Research Group

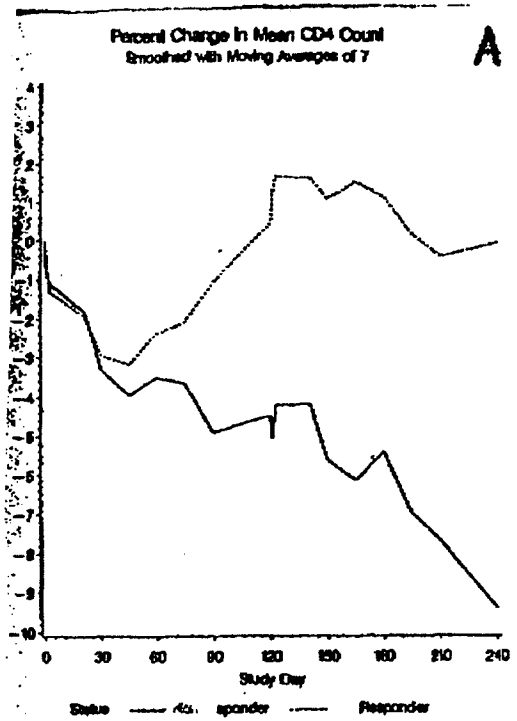
ATTACHMENT 1

Re-Analysis of NEJM Fig. 4. [responders vs. non-responders; as defined by RRR in the NEJM article].

Used actual raw patient CD4 data that was used in the NEJM Fig. 4 Statistical methods used outlined in McCarthy Army statement and earlier McCarthy memos.

	t-test	Wilcoxon
Slope (uses all data points)	p=0.13	p=0.14

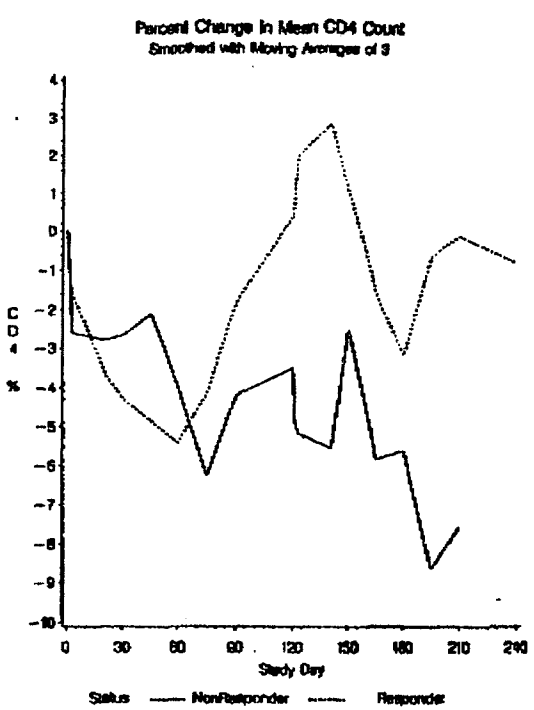
A


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 7
CD4 %
Study Day
Status — NonResponder — Responder

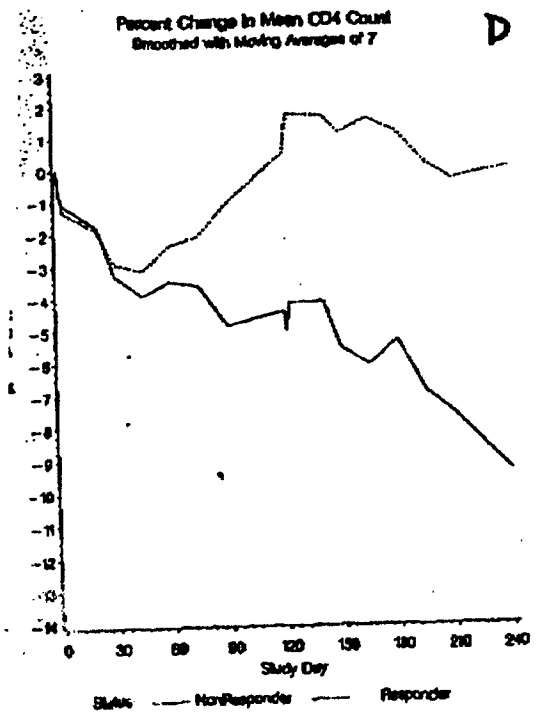
B


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 5
CD4 %
Study Day
Status — NonResponder — Responder

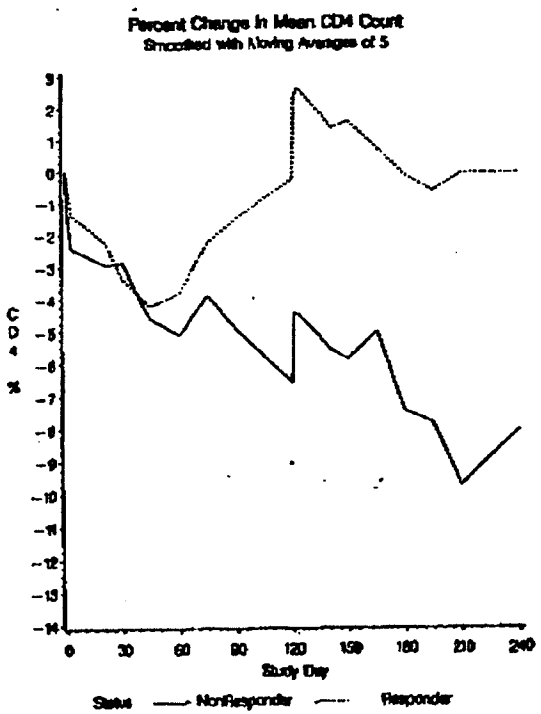
C


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 3
CD4 %
Study Day
Status — NonResponder — Responder

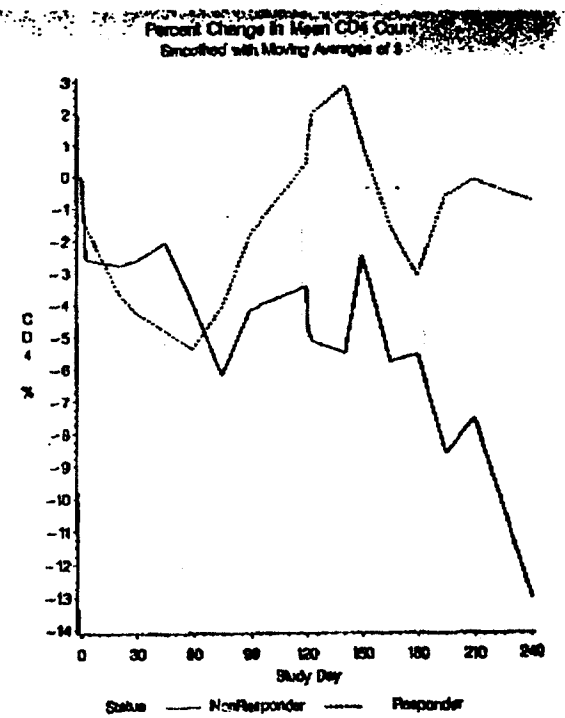
D


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 7
Study Day
Status — NonResponder — Responder

E


Percent Change In Mean CD4 Count
Smoothed with Moving Averages of 5
CD4 %
Study Day
Status — NonResponder — Responder

F


Study Day
Status — NonResponder — Responder

ATTACHMENT 3

ALTERATION OF HIV SPECIFIC DNA AND RNA

	HIV PROVIRAL DNA			FULL LENGTH HIV RNA**		
	1/2 log increase*	1/2 log decrease or no change*	1/2 log decrease	1/2 log increase^	1/2 log decrease or no change^	1/2 log decrease
Natural History (n=19)	47%	53%	16%	47%	53%	11%
rgp160 Vaccinees (n=15)	0%	100%	60%	7%	93%	33%

* p=0.003 Fisher Exact (2-sided)
^ p=0.02 Fisher Exact (2-sided)
**Wilcoxon (Mann-Whitney) p < 0.05 (two-tailed)

[redacted]

HIV DNA and RNA ASSESSMENT
NATIONAL HISTORY AND VACCINE THERAPY (RGP160)
VOLUNTEERS WITH EARLY HIV INFECTION
(24 MONTH FOLLOW-UP)

	Time	DNA* Arith Mean	DNA* Geom Mean	DNA* Range	RNA** Arith Mean	RNA** Geom Mean	RNA** Range
Natural History (n=19)	0	743	227	(27-5673)	422	136	(5-2075)
	1 year	1664	322	(9-17,331)	1967	319	(13-37,384)
	2 year	3055	476	(26-35,659)	3882	472	(25-31,625)
rgp160 Vaccinees (n=7)	0	297	153	(40-1344)	1338	322	(5-4485)
	1 year	268	129	(21-1246)	518	229	(6-1088)
	2 year	122	50	(11-610)	634	250	(18-1654)

* Wilcoxon (Mann-Whitney) $p < 0.1$ (one-tailed); ** Wilcoxon (Mann-Whitney) $p < 0.05$ (one-tailed)

ATTACHMENT 4

was counterintuitive. Yet we have demonstrated that in the setting of early HIV infection this assumption is incorrect. The mechanism enabling postinfection vaccination to broaden HIV-specific immune responses remains to be determined. It is possible that postinfection vaccination results in qualitative difference in HIV envelope epitope presentation and antigen processing, thereby resulting in an altered immune response. Altered immune responses may be the result of differences in viral genotype, posttranslational modification of viral protein, alteration in protein conformation, differences in protein formulation, or a result of the immunization process itself. If, in fact, some of these proposed mechanisms are involved in the expansion of the immune response in chronic HIV infection, it is likely that this therapy can be applied to other chronic disease processes.

Alternatively, the difference may be due to a quantitative difference in the efficacy of presentation and processing of envelope epitopes between naturally produced proteins and vaccine administered protein. For example, gp120 bound to CD4 would influence the efficacy of antigen presentation via antigen processing cells. It is also possible that the cellular pathways within antigen processing cells are altered or inefficient as a consequence of HIV macrophage infection. If strictly a quantitative phenomenon, the potential for extrapolation toward broader application in medicine may be premature. Careful investigation into the mechanism of why vaccine therapy with rgp160 is capable of redirecting augmenting the host anti-HIV immune responses undoubtedly will reveal insight into the mystery of human immunology.

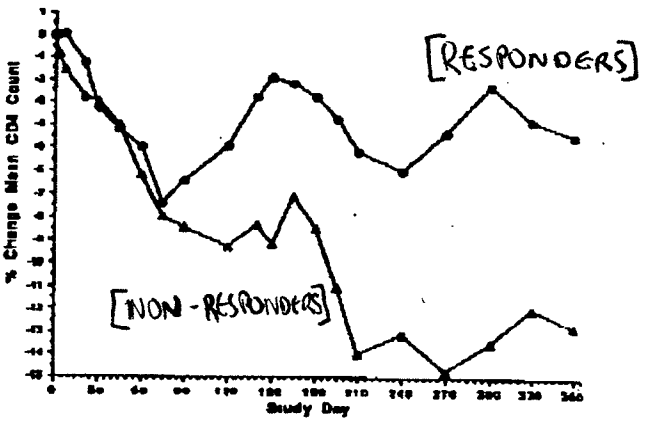


FIG. 5. Comparison of rgp160 humoral responders versus humoral nonresponders post vaccine therapy. The figure depicts the percent change from baseline mean CD4 count of volunteers who received rgp160 vaccine therapy. Circles represent mean CD4 change over one year in volunteers who developed novel anti-envelope antibody responses. Triangles represent mean CD4 change over same year in volunteers who failed to develop new anti-HIV envelope antibodies post vaccination. Study Day 0 is defined as the day of initial vaccination. Novel antibody was associated with less change in mean CD4 counts over the one-year period. Epitope-specific analysis is ongoing.

WHAT IMMUNE RESPONSES ARE RESPONSIBLE FOR EFFECTIVE POSTINFECTION IMMUNOREGULATION AND VIRAL REPLICATION CONTROL? CONTINUATION OF ASSAY DEVELOPMENT TO ASSESS IMMUNOREGULATORY MECHANISM

One objective of this vaccine therapy study was to facilitate the development and validation of an in vitro immune response assay of in vivo immunoregulatory relevance. To date no such assay exists for HIV infection. Although vaccine therapy with rgp160 induces novel immune responses, the biological consequences of each of these responses is unknown. Unlike natural history descriptive studies, postinfection vaccine therapy studies allow causal relationships to be ascertained. For example, Figure 5 demonstrates the relationship between the induction of new anti-gp120 antibody responses and a clinical surrogate of HIV disease progression, CD4 decline. Recently we published the development of quantitative RNA and DNA polymerase chain reaction assays to measure copy number of viral RNA and the expression ratio (i.e., copy number of genomic viral RNA/proviral DNA) in volunteers' peripheral blood mononuclear cells.[17] Application of this technique is in progress and will provide the opportunity to characterize the induction of specific immune responses which result in alteration of HIV genomic RNA production.

We continue to develop new assays to describe HIV envelope immunologic responses. Recently we explored the application of Biacore instrumentation relative to our interest in binding antibody affinity.[18] This technology should facilitate the assessment of protein conformational changes as a consequence of antibody or receptor binding. In addition, we are utilizing Pepscan techniques to fine map antibody specificity and have recently explored techniques to evaluate conformational antibodies directed against the HIV envelope.[19]

We are also exploring neutralization assays which will have in vivo relevance. A myriad of factors such as the viral genotype used, cell type used to propagate viral stocks, target cells involved in the neutralization assay, what parameters are measured to assess viral production, time course of assay and fluctuation of assay endpoint will impact on the clinical utility of neutralization assays. Present standard HIV neutralization assays utilizing prototype isolates and/or cell line targets are suboptimal in this regard. Recently our group and others have developed a neutralization assay using the patients' own isolate, propagated in primary PBMC, assayed in primary PBMC, utilizing molecular endpoints of viral replication.[20] This assay is currently being applied to assess alterations in neutralization activity incuded by vaccine therapy with rgp160.

FUTURE DIRECTIONS

In light of the encouraging Phase 1 trial results, the Department of Defense began a double-blinded, placebo-controlled Phase 2 trial in November 1990 designed to assess the clinical efficacy of rgp160 in the treatment of patients with early HIV infection. This trial is currently ongoing and an assessment of efficacy potential should be forthcoming over the next several years.