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Joan Claybrook, President

June 7, 1994

Representative Henry Waxman
Subcommittee on Health and the Environment
Committee on Energy and Commerce
U.S. House of Representatives
2415 Rayburn House Office Bldg.
Washington, DC 20515

Dear Representative Waxman:

We are writing to request that your Subcommittee hold a hearing, as soon as possible, to investigate charges of grave impropriety committed by U.S. Department of Defense' AIDS researchers. We have obtained internal memoranda, not previously made public, from the Department of Defense that allege a systematic pattern of data manipulation, inappropriate statistical analyses and misleading data presentation by Army researchers in an apparent attempt to promote the usefulness of the GP160 AIDS vaccine (VaxSyn; MicroGeneSys, Meriden, Connecticut), which is intended to prevent the progression of disease in persons with HIV infection. The Phase I and Phase II studies in which this alleged misconduct occurred were conducted by researchers at the Walter Reed Army Institute of Research (WRAIR), led by Lt. Col. Robert Redfield, M.D., Chief of the Department of Retroviral Research, and misleading results from these trials were reported in a variety of scientific fora, including the *New England Journal of Medicine* in June 1991, the journal *AIDS Research and Human Retroviruses* in June 1992 and the annual International AIDS Conference in Amsterdam in July 1992. In addition, overstated conclusions have been presented on two occasions at hearings before your Subcommittee.

Meeting on October 23, 1992 to discuss the allegations by two Air Force research physicians (see below) of scientific misconduct by Dr. Redfield, a subcommittee of the Institutional Review Committee at the Wilford Hall U.S. Air Force Medical Center, San Antonio, Texas reached the following conclusion (see Attachment 1):

The committee agreed the information presented by Dr. Redfield seriously threatens his credibility as a researcher and has the potential to negatively impact AIDS research funding for military institutions as a whole. His allegedly unethical behavior creates false hope and could result in premature deployment of the vaccine. The need for Phase II studies, which stand to answer questions raised in this controversy, could also come into question.

That meeting was called to review an October 21, 1992 memorandum (see Attachment 2) from Maj. Craig W. Hendrix, M.D., Director of the HIV Program in the Air Force, and Col. R. Neal Boswell, M.D., Associate Chief of the Division of Medicine in the Air Force, to Col. Donald Burke, M.D., Director of the Division of Retrovirology at WRAIR and Dr. Redfield's immediate supervisor. The memorandum decried "The problem of misleading or, possibly, deceptive presentations by Dr. Redfield, which overstate the GP160 Phase I data . . ." and recommended that the following action be taken:

(1) publicly correct the record in a medium suitable for widespread dissemination to our civilian scientific colleagues;

(2) censure Dr. Redfield for potential scientific misconduct which should at least include temporarily suspending his involvement on the current immunotherapy protocols; and

(3) initiate an investigation by a fully independent outside investigative body, such as the Office of Scientific Integrity [now the Office of Research Integrity] of the NIH, to evaluate the facts of the case and recommend appropriate actions.

Senior Department of Defense scientists have known of this misconduct since at least October 1992, and Dr. Redfield has acknowledged that his analyses were faulty on at least three occasions to internal Department of Defense audiences (the earliest admission was on August 28, 1992). A year and a half after Drs. Hendrix and Boswell made their requests (which were endorsed by the Directors of the Clinical HIV Programs in the Army and Navy, Col. Charles Oster and Capt. Walter Karney, respectively), none of their three demands has been met. Instead, the faulty analyses have never been publicly retracted, Dr. Redfield continues to conduct trials of GP160 and only an internal Army investigation has been conducted. That "informal investigation," by the Army's Col. Harry Dangerfield, concluded that "Evidence does not support the allegations of scientific misconduct." The recommendations of the report were:

1. *There is no requirement for adverse actions.*
2. *In fairness to LTC Redfield, the HIV Research Program, the Army and the scientific community, a press release correcting the record is warranted.*
3. *Measures to enhance the effectiveness of communication are warranted.*

Col. Dangerfield's investigation lends new meaning to the term "whitewash." Massive parts of the testimony of key figures have been whited out in documents obtained through the Freedom of Information Act, purportedly because the excised section "would have a chilling effect on open agency communications and/or is personal in nature which, if released, would result in an invasion of an individual's personal privacy." We have attached (see Attachment 3) the full version of the statement to Col. Dangerfield by Dr. William McCarthy, Director of Biostatistics for the Henry M. Jackson Foundation, a non-profit foundation created by an act of Congress to work with Department of Defense researchers, and have indicated which portions have been removed in the copy obtained through the Freedom of Information Act. The specifics of Dr. McCarthy's concerns have consistently been whited out.

The testimonies of others who questioned Dr. Redfield's analyses have been similarly edited. Only the introductory paragraphs and signatures remain from a ten page statement by Dr. Hendrix and a three page statement by Dr. Boswell.

Hundreds of HIV-infected persons have been enrolled in extremely expensive trials at WRAIR as well as in Massachusetts, Connecticut, New York City, Montreal and Sweden. While there are obvious similarities between the issues raised here and the recent revelations that investigators in the breast cancer trials failed to retract results based on incorrect data, the scientific misconduct in this case is more egregious in that it significantly altered the study results and may have resulted in hundreds of people being given the vaccine.

As noted above, findings from the Phase I trial have been presented in the *New England Journal of Medicine*, before your Subcommittee on two occasions, in the journal *AIDS Research and Human Retroviruses*, at the Amsterdam International AIDS Conference in July 1992 and at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Summaries of the misleading aspects of these publications and presentations are presented below.

1. New England Journal of Medicine

To our knowledge, the misconduct in this case dates back to a June 13, 1991 issue of the *New England Journal of Medicine* (see Attachment 4). In that article, Dr. Redfield and his colleagues presented data purporting to show that the CD4 cell count (an index of damage to the immune system in persons infected with HIV) remained stable among those who responded to the vaccine, while it declined in a control group of non-responders to the vaccine, a result characterized in the paper as "encouraging."

In the aforementioned statement of Dr. William McCarthy to the Army's internal investigation -- in one of the many portions whited out in the publicly available version -- Dr. McCarthy, described his re-analysis using more appropriate statistical methods of the data in the *New England Journal* article (see Attachment 3):

Using this approach my department determined that there was no statistical[ly] significant difference between the responders and the non-responders CD4 count longitudinal profiles.

Reviewing these published data in the *New England Journal* and subsequent oral presentations, Drs. Hendrix and Boswell stated in their October 21, 1992 memo (see Attachment 2):

Data analysis has been sloppy or, possibly, deceptive with use of inappropriately chosen "control" groups, unorthodox statistical methods that abuse the data to come up with the desired CD4 trend conclusions, failure to include appropriately performed analyses that fail to support the desired conclusion and badgering of statisticians and colleagues by Dr. Redfield, sometimes successfully, to agree to data analyses against their better professional judgment.

2. Congressional Testimony

On June 6, 1991, Dr. Redfield appeared before your Subcommittee and testified, referring in part to the same data presented in the *New England Journal* article, that "the individuals that have been immunized appear not to have a fall in their CD4 cells as opposed to historical controls."

Dr. Redfield appeared before your Subcommittee again on February 24, 1992, this time accompanied by Dr. Burke and Mr. Sheppard Smith, President of Americans for a Sound AIDS Policy (ASAP), a group that raises funds for HIV research and medical care. At that time, Dr. Redfield was Chairman of ASAP's Advisory Board and Dr. Burke served on the Executive Committee; both Lt. Col. Deborah Birx, M.D., Dr. Redfield's assistant, and Col. (Ret.) Edmund Tramont have also served on the Board.

Dr. Burke stated that "What was most remarkable in this study was that blood counts of the CD4 positive T helper cells remained stable over the 1 year period of observation in study patients with boosted immunity." Dr. Burke also indicated that \$10 to \$20 million would be needed to conduct the requisite follow-up studies. Dr. Redfield indicated that Army researchers were "within 12 to 19 months" of determining whether GP160 could prevent progression of disease. Mr. Smith, who is not a scientist by training, added that "Undoubtedly, any military witnesses today will understate the significant advances being made in regard to vaccine therapy at Walter Reed Army Institute of Research."

In October 1992, several months after the Amsterdam AIDS meeting (see below), Congress appropriated \$20 million for a Phase III trial of VaxSyn to be conducted by the Army. That appropriation was modified on January 4, 1994 after it generated considerable controversy, including opposition from the NIH, the Food and Drug Administration and leading AIDS researchers. Army researchers will now use the funds for a variety of different types of HIV vaccine research.

3. AIDS Research and Human Retroviruses

In June 1992, Drs. Redfield and Birx published an article in the journal *AIDS Research and Human Retroviruses* (see Attachment 5) that stated:

Although the study was not designed to assess efficacy, CD4 counts were carefully monitored throughout the trial. It is extremely intriguing that at the time of analysis original vaccine responders experience 2.8% decline [in CD4 counts], and all trial volunteers 8.5% in contrast to historical natural history experience of a 26.1% decline. These data demonstrate long-term (2-3 year) safety and hint at clinical benefit.

The implication was that the decline in CD4 counts in non-vaccinated control patients was averted in those getting the vaccine.

4. International AIDS Conference

On July 21, 1992, Dr. Redfield presented updated data from the GP160 Phase I study at the International AIDS Conference in Amsterdam. Dr. Redfield presented slides (see Attachment 6) purportedly demonstrating statistically significant decreases in the amount of HIV in the patients' blood (viral load) among vaccinees compared to a control group. In a presentation at the Amsterdam Conference, later aired on CBS TV, Dr. Redfield described the reported decrease in the viral load among vaccine recipients compared with people not getting the vaccine: "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 (see Attachment 3), the data presented for the vaccinated patients were

for only 7 subjects in one slide and 15 subjects in another. Dr. Redfield was also quoted in the *New York Times* (July 26, 1992) and the *Wall Street Journal* (July 15, 1992) as saying that there was stabilization of the CD4 cell count among vaccine recipients.

It was the July 1992 Amsterdam presentation that first raised questions about the Phase I trial data. In August 1992, Dr. William McCarthy and Lt. Col. John Brundage, head of Epidemiology at WRAIR, were called in to separately reanalyze the raw Amsterdam data but were unable to replicate Dr. Redfield's results for the viral load.

Dr. McCarthy also informed Dr. Burke that "the CD4 count longitudinal profiles of the GP160 Phase I patients were not stabilizing" (see Attachment 3). Dr. Redfield agreed at a 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. That meeting was attended by Drs. Redfield, Burke, Brundage, and McCarthy as well as Dr. Maryanne Vahey, who had performed the viral load analyses for Dr. Redfield and who had first questioned the validity of the Amsterdam presentation. At that meeting, Dr. Redfield agreed that an upcoming poster presentation by Dr. Vahey at the Advances in AIDS Vaccine Development conference in Chantilly, Virginia on August 31, 1992 should include the data on *all* subjects and the correct statistical analyses, without the inappropriate control group.

However, on August 24, 1992, shortly before the Chantilly meeting was to occur, Dr. Vahey received a telephone call from Mr. Sheppard Smith of ASAP. Drs. Hendrix and Boswell reported the following in their October 21, 1992 memorandum (see Attachment 2):

According to Dr. Vahey, Mr. Smith had intimate knowledge of the GP160 Phase I data and offered detailed suggestions for how Dr. Vahey should present the incomplete data with the control group, coincidentally as Dr. Redfield had done in Amsterdam, to favor further development of the vaccine. He also insisted that she needed to know of the increasing pressures on her due to: (1) the millions of dollars at stake, (2) Army-NIH vaccine competition, and (3) upcoming congressional testimony [on] GP160 vaccine studies. We are suspicious of Mr. Smith's access to GP160 data, his involvement at the most basic level of data analysis on this study, and his motivations in raising issues of financial and congressional pressure which are scientifically immaterial and have, on the surface, the appearance of a very gross impropriety.

To our knowledge, Dr. Vahey did not alter her presentation as a result of the phone call.

5. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)

On at least two occasions after Dr. Vahey's Chantilly presentation, Dr. Redfield again admitted before the Department of Defense's researchers that the presentation in Amsterdam had been incorrect and misleading. Following these admissions, however, Dr. Redfield made yet another misleading presentation at the ICAAC meeting in Anaheim, California on October 13, 1992 that, according to Drs. Hendrix and Boswell, "continued to present selected patients and made partially true statements to maintain the misleading message." In the published abstract from the meeting, Dr. Redfield and his colleagues report that:

the reduction of in vivo HIV expression supports an antiviral effect of this therapeutic strategy. (see Attachment 7)

Phase II Trials

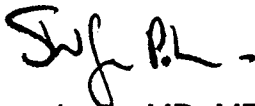
In addition to the concerns regarding the Phase I trials described above, Drs. Hendrix and Boswell also raised questions about the Phase II trial of GP160 being conducted at WRAIR and for which Dr. Redfield was also principal investigator. Although researchers are not supposed to know whether patients are in the vaccine or control group (blinding), according to Drs. Hendrix and Boswell "An unblinded laboratory investigator has been seeing GP160 patients for research visits" and "unblinded data on current Phase II patients has been presented to other clinical investigators on the study."

We strongly urge you to hold a hearing to further explore these issues and to request a full investigation of these events by a truly independent body. In addition to this investigation, a censure of all military and other personnel who are found to have engaged in scientific misconduct is critical. The scientific record, including publications and presentations, should be immediately corrected by requiring Dr. Redfield and his colleagues to issue prominent retractions.

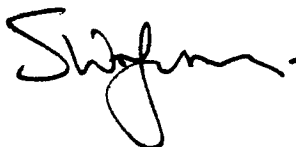
The events described here illustrate the increased potential for scientific misconduct when fame, financial reward and even a Nobel Prize await the discoverer of an effective HIV vaccine and suggest the need for special monitoring of research in this area. These incentives appear to have produced a campaign to promote GP160

that bears more resemblance to market research than it does to objective scientific research. The real tragedy here is that hundreds of HIV-infected persons have been recruited to get this vaccine, perhaps as a result of these misleading analyses. As Drs. Hendrix and Boswell stated at the end of their memorandum, "We cannot continue to deceive."

Sincerely,

A handwritten signature in black ink, appearing to read "Peter Lurie".

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

A handwritten signature in black ink, appearing to read "Sidney M. Wolfe".

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

DEPARTMENT OF THE AIR FORCE
WILFORD HALL USAF MEDICAL CENTER (ATC)
LACKLAND AFB, TEXAS 78236-5300

ATTACHMENT 1

FROM: SGS (Mrs Whitaker/7143)

23 Oct 92

SUBJ: Minutes of the Institutional Review Committee (IRC) Subcommittee
on Potential Scientific Misconduct in GP160 Phase I Immunotherapy Study

TO: WHMC IRC
SG-2

1. PLACE: Clinical Investigation Conference Room
2. DATE AND TIME OF MEETING: 23 Oct 92, 0800 hours

3. ATTENDANCE:

a. Members:

Col John H. Clask, BSC	SGS	Chairman
Lt Col Frank J. Criddle, MC	SGH	Dep. Dir., Hospital Services Dir.
Maj Paul M. Dankovich, JD	SGJ	Dir, Medical Law
Capt Steven G. Davis, BSC	SGHDP	Chief, Clinical Pharmacy
Dr Clifford A. Butzin, PhD	SGS	Consultant (non-voting member)
Mr James M. Wilbourn, GS-12	LTC/XR/3	Research Psychologist
Mrs Nancy K. Whitaker, GS-6	SGS	Recorder (non-voting member)

b. Visitors:

Col R. Neal Boswell, MC	SGHM	Assoc. Chief, Div of Medicine
Maj Craig W. Hendrix, MC	SGHMI	Director, HIV Program
Capt Stewart R. Wirebaugh	SGHDP	Clinical Pharmacist
Dr George Kelling, PhD	SGPA	Historian/Public Affairs Rep

4. DISCUSSION:

a. This subcommittee was convened under the auspices of AFR 169-8, "Human Use in Clinical Investigations," and WHMC MCR 169-11, "Scientific Fraud and Misconduct," to address an alleged incidence of misconduct by Lt Col Robert Redfield, an Army researcher involved in the Phase I GP160 Immunotherapy Study, assigned to the Walter Reed Army Institute of Research (WRAIR). Maj Hendrix presented a chronological overview of events leading up to a letter he and Col Boswell forwarded to Col Donald Burke, Director of WRAIR, on 21 Oct 92. Maj Hendrix's point paper and a copy of the letter are attached. For clarification, the following five groups involved in the Military Medical Consortium for Applied Retroviral Research (MMCARR) and key people are identified:

(1) Walter Reed Army Medical Center, Col Charles N. Oster, Chief, Infectious Disease Service

(2) Wilford Hall Medical Center Clinical Unit, Maj Craig Hendrix, Director, HIV Program

(3) National Naval Medical Center, Capt Walter Karney, Manager, HIV Navy Program

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IRC Subcommittee, 23 Oct 92

(4) Walter Reed Army Institute of Research (WRAIR), Col Don Burke, Director; Lt Col Robert Redfield, Principal Investigator of Phase I Study; Dr Maryanne Vahey, researcher in Dr Redfield's lab; Dr John Brundage, Epidemiologist.

(5) Henry M. Jackson Foundation Lab; Dr Bill McCarthy, Statistician; Dr John Brundage, Epidemiologist.

b. The committee discussed Maj Hendrix's allegation that Dr Robert Redfield may have either misled or deceived the scientific community in several presentations of the GP160 Phase I Immunotherapy Study. His 21 Oct 92 letter makes three recommendations:

(1) There must be widest possible public correction of the record to show that findings presented by Dr Redfield are premature and/or unsubstantiated.

(2) Dr Redfield should be censured for scientific misconduct, if this is proven.

(3) An independent investigation of the Phase I study should be conducted.

CONCLUSION: It was noted that no Wilford Hall patients or investigators have been involved in the Phase I study in question. The committee agreed the information presented by Dr Redfield seriously threatens his credibility as a researcher and has the potential to negatively impact AIDS research funding for military institutions as a whole. His allegedly unethical behavior creates false hope and could result in premature deployment of the vaccine. The need for Phase II studies, which stand to answer questions raised in this controversy, could also come into question.

RECOMMENDATIONS/ACTION: The committee voted unanimously to take the following action:

(1) Through the chain of command, address the fact that the triservice agreement stating a HMJF statistical group must evaluate all data presented for oral presentation or publication has not been adhered to. Evaluations must be done with no exceptions; and

(2) address the fact that papers/data from any MMCARR source must be reviewed in-house prior to release (this also has not been adhered to).

(3) Dr Hendrix and Dr Boswell should be officially tasked to conduct a fact-finding visit to WRAIR along with Col Oster (USA) and Capt Karney (USN). Based on their findings, they may recommend an audit by an outside agency. A full disclosure in the form of a written report should be presented to the IRC and to HQ AFMOA/SG. If an outside evaluation is requested, the agency should attempt to gather video/audiotapes of presentations made in public settings by Dr Redfield.

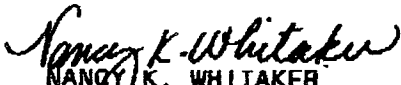
(4) While the focus of investigation is the Phase I GP160 study, the appearance of impropriety in one study raises into question the entire process of data analyses and presentation for all protocols which have been activated at WHMC through the MMCARR mechanism. The findings of internal or external review should be considered by the WHMC IRC in the context of potential impact on all consortium protocols.

(5) Dr Hendrix should be appointed in writing as principal representative from WHMC to the MMCARR, and for all HIV-related issues. The committee believes Dr Hendrix should be officially commended for his concern and for his thorough and timely response to this issue.

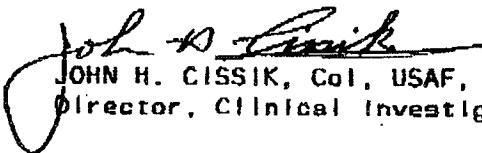
(6) WHMC should continue to participate in the GP160 Phase II studies, since it is anticipated these studies will answer questions about efficacy of the GP160 vaccine.

FOLLOW-UP: OPEN: 27 Oct 92, OPR: Maj Hendrix

5. ADJOURNMENT: 0945 hours


NANCY K. WHITAKER
Protocol Coordinator
Clinical Investigations

- 3 Atch
1. Point Paper
2. SGHMI-H Ltr, 21 Oct 92
3. Science article, 9 Oct 92


JOHN H. CISSIK, Col, USAF, BSC
Director, Clinical Investigations

DEPARTMENT OF THE AIR FORCE
 WILFORD HALL USAF MEDICAL CENTER (ATC)
 LACKLAND AIR FORCE BASE TX 78238-5300



REPLY TO: SGHMI-H/Maj Hendrix (DSN 554-7897)

21 Oct 92

SUBJECT: GP160 phase I Immunotherapy Data Presentation

TO: Col Donald Burke
 Director, Division of Retrovirology
 WRAIR

1. The problem of misleading or, possibly, deceptive presentations by Dr. Redfield, which overstate the GP160 phase I data, persists despite several efforts on your part to correct the problem. Last week, at the 32nd Annual ICAAC meeting in Anaheim, CA, Dr. Redfield again presented data in an incomplete and misleading fashion, despite assurances to the MMCARR in several recent meetings that he understood his past presentations to be in error and that he would refrain from repeating that error. If these actions are an intentional deception, it is an error of the most serious kind in science that betrays the trust of colleagues, patients and sponsors. Accordingly, we insist that further action be taken immediately to: (1) publicly correct the record in a medium suitable for widespread dissemination to our civilian scientific colleagues, (2) censure Dr. Redfield for potential scientific misconduct which should at least include temporarily suspending his involvement on the current immunotherapy protocols, and (3) initiate an investigation by a fully independent outside investigative body, such as the Office of Scientific Integrity of the N.I.H, to evaluate the facts of the case and recommend appropriate actions. Over the past two months, as you know, we have discussed all of the background issues summarized below with the Directors of the Clinical HIV Programs in the Army and Navy, Colonel Charles Oster and Captain Walter Karney, respectively. Both officers support our conclusions and the three recommended actions.

2. The most serious example of potential scientific misconduct is related to a presentation of GP160 phase I data at the AIDS conference in Amsterdam on 21 JUL 92. Dr. Redfield presented data which showed a statistically significant fall in viral burden in selected GP160 phase I vaccine recipients compared to a historical control group. That analysis cannot be supported by the data as you are well aware. On 31 JUL 92, Dr. Maryanne Vahey, who performed the viral burden assays and first questioned Dr. Redfield's Amsterdam talk, presented to Dr. Redfield's laboratory group data on all 19 vaccine recipients that showed neither stable T-helper cells nor a fall in viral burden. She also rejected as inappropriate the "control" group Dr. Redfield personally selected. At your request, Dr. Bill McCarthy and Dr. John Brundage performed their own analyses of the data, and those analyses supported Dr. Vahey's conclusions. In fact, they could not reproduce Dr. Redfield's analysis even by selecting only some patients and they rejected the use of the control group. At your urging and at the insistence of the Air Force and Navy HIV programs, Dr. Redfield agreed in a meeting on 28 AUG 92 that the complete data and correct analyses, without the discredited control group, should be presented. Dr. Vahey then presented the data at the Chantilly, VA vaccine meeting on 31 AUG 92. On two subsequent

occasions (15 and 24 SEP 92) in the presence of MMCARR audiences, Dr. Redfield admitted making mistakes in data analysis and in making misleading presentations. He also agreed to changes in procedures to prevent the same mistakes from occurring in the future. On October 13, however, at the 32nd Annual Meeting of the ICAAC in Anaheim, CA, Dr. Redfield continued to present selected patients and made partially true statements to maintain the misleading message. When Maj Hendrix suggested publicly that Dr. Redfield's conclusions were "premature" based on the wide variability of the viral burden assay (an observation with which Dr. Redfield agreed when Dr. Deborah Birx first raised it in the 15 SEP 92 meeting) he reasserted his conclusion that the data suggest an antiviral effect of the vaccine. He failed to seize the opportunity Maj Hendrix afforded to clarify his message. Furthermore, he failed to correct his published abstract which states that the viral burden goes down after GP160 vaccination which, again, the data do not support. He clearly has not received the message of the 28 August and 15 September meetings, among others, or he has consciously chosen to ignore the message denying the serious consequences of this scientific misconduct.

3. Overselling the GP160 phase I data may date back as early as the New England Journal of Medicine publication (N Engl J Med 1991: 324:1677-84). Oral presentations of the phase I continuation data over the last year and a half have repeatedly shown only a few selected "home run" patients. The data from the entire study group has not been well represented by these selected patients. Data analysis has been either sloppy or, possibly, deceptive with use of inappropriately chosen "control" groups, unorthodox statistical methods that abuse the data to come up with the desired CD4 trend conclusions, failure to include appropriately performed analyses that fail to support the desired conclusion and badgering of statisticians and colleagues by Dr. Redfield, sometimes successfully, to agree to data analyses against their better professional judgement. There are many examples of presentation of the data that have been couched in terms of safety, but delivered in a manner that communicates a message almost universally received as data supportive of efficacy, when the facts do not support this. This message can be subtle with words chosen so carefully that they are not technically false, but give the observer a misleading impression. As recently as the 15 SEP 92 meeting and on several previous occasions, it has been made clear to Dr. Redfield that the nature of the "efficacy" message received is significantly different than the "safety" message purportedly intended. Such potentially deceptive presentations persist, however, as evidenced by the ICAAC presentation last week.

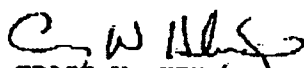
4. Additional facts create at least the perception that the integrity of the GP160 phase II trial may also have been diminished. An unblinded laboratory investigator has been seeing GP160 patients for research visits and has, at least on one occasion, altered previous Walter Reed staging designations (telephone conversation with Col Oster 4 Sep 92). Additionally, despite Maj Hendrix's written and verbal protestations to Dr. Redfield, unblinded data on current phase II patients has been presented to other clinical investigators on the study. We feel strongly that both of these events may be perceived as potential sources for significant bias in the phase II study, if not protocol violations.


5. Another serious concern is also one of perceptions. Mr. Sheppard Smith, President of Americans for a Sound AIDS Policy (ASAP) has made very frequent

visits to Dr. Redfield at the WRAIR Gude Drive laboratory which we assume are related to Dr. Redfield's position as Chairman of ASAP. Mr. Smith also contacted Dr. Vahey prior to her 31 AUG 92 presentation of GP160 phase I data which was to be the first complete public presentation of the data in a non-selective, appropriately analyzed fashion. According to Dr. Vahey, Mr. Smith had intimate knowledge of the GP160 phase I data and offered detailed suggestions for how Dr. Vahey should present the incomplete data with the control group, coincidentally as Dr. Redfield had done in Amsterdam, to favor further development of the vaccine. He also insisted that she needed to know of the increasing pressures on her due to: (1) the millions of dollars at stake, (2) Army-NIH vaccine competition, and (3) upcoming congressional testimony of GP160 vaccine studies. We are suspicious of Mr. Smith's access to GP160 data, his involvement at the most basic level of data analysis on this study, and his motivations in raising issues of financial and congressional pressure which are scientifically immaterial and have, on the surface, the appearance of a very gross impropriety.

6. Finally, let us speak clearly as to our motivations. The Wilford Hall Medical Center site has been a major contributor to the MMCARR's Immunotherapy MAP consistently over the three years of our involvement in MMCARR. At the Operation and Plans Committees our occasional criticisms have been open and honest in an effort to improve the science of the Immunotherapy MAP and to be sure that program priorities were being met. Once these program priorities were decided, Wilford Hall has been aggressive in enrolling patients at our site on Triservice protocols and equally active in referring patients to the Walter Reed site for protocols conducted only at that site. In fact, in the recent GP120 phase I study, Wilford Hall referred more patients to Walter Reed than any other military center outside of Walter Reed itself. Our support for the program as a whole is unflinching as our performance in the past should clearly demonstrate. Our interest in this matter is primarily to see that information is presented as factually as possible so that the creditability of our research program will be maintained and the science of HIV will be furthered.

7. We regret that the problem must be raised to this level, but actions taken to date have failed to resolve the problem. The scientific credibility of the entire MMCARR is at risk and is already being questioned by those outside our organization. Severe, painful steps must be taken lest we dishonor the honest labors of so many colleagues and patients within our research consortium. We cannot continue to deceive.


CRAIG W. HENDRIX, Maj, USAF, MC
Director, HIV Program


R. NEAL BOSWELL, Col, USAF, MC
Associate Chief, Division of Medicine

cc: HQ USAF/SG
WHMC/SG-1
Col Salvado
WRAIR/Director
Col Oster
WRMC/Ch., I.D. Serv.
Capt Karney
NNMC/Mgr, HIV Navy Prog.

STATEMENT

ATTACHMENT 3

OF

William F. McCarthy, Ph.D.

1015-1150

7 December 1992

portions
"whited out" in
public version

COL Dangerfield opened the meeting with an explanation of the process for the informal investigation and reviewed the tasks for which he was responsible to determine facts. He then reviewed the allegations that had prompted the investigation.

I responded to his question regarding a statement made by some individual that LTC Redfield and I never really got along with one another. I stated that the statement was not true; that Redfield had told a number of individuals that he was pleased with my work; that he was very supportive of me and my work. COL Dangerfield then asked me to relate the events involved with the statistical analysis of the gp160 RV21 Phase I trial viral burden data.

The initial meeting with LTC Redfield and Dr. Vahey was on 11 August 92 in his office; this was the first time that I had met Dr. Vahey. They gave me the data for analysis and LTC Redfield told me how he wanted the data to be analyzed. I told him I would review his design (method of analysis) and get back to him. LTC Redfield gave me the impression that he had performed the analyses for the Amsterdam presentation. After the meeting, Dr. Vahey showed me some of the analyses she had done on the PCR assay data and she stated that she provided the results of the analyses to LTC Redfield before he went to Amsterdam. [At a later date, after I had completed and presented the findings of all my analyses, Dr. Vahey told me that before Amsterdam she told LTC Redfield that she had done statistical analyses on the PCR copy data and found no significant findings.]

On 13 August 92, I held a meeting with Dr. Ng (my senior biostatistician) and Dr. Vahey at the main office of the Jackson Foundation at 1401 Rockville Pike to critique the LTC Redfield design (method) for the analysis. Dr. Ng and I together had concluded that LTC Redfield's design (method) for the analysis was not appropriate from a research methodological as well as a statistical point of view. At this meeting Dr. Ng and I described what we considered an appropriate research methodological and statistical approach for the analysis.

The following day LTC Redfield called me. He was very angry that I would not do the analysis his way. He was very abusive and basically stated that statisticians just get in the way of things, that I didn't know anything about this subject, that he did; that the theory of "the big O (obvious)" applied in this situation [He explained the theory of the big O as follows: that if you see results with the first four or five patients that is all you need to see in order to demonstrate that you have an effect; it is obvious to everyone that there is an effect]. Although I did not appreciate his comments, I did not respond in kind but remained objective and stood firm about using the method of analysis which

I felt was appropriate. He stated that if I did not do the analysis his way he would go to someone else who would. I reported the incident to Mr. Lowe. Dr. Vahey latter told me she was present with LTC Redfield (in his office) when he called me ; that she overheard the entire conversation and shortly thereafter she told COL Burke about the conversation.

Shortly after this, I talked to MAJ John McNeil who worked for LTC Brundage at WRAIR to discuss the PCR data analysis issues, e.g., who did the analysis for Amsterdam, etc. It was my impression from him that LTC Redfield had told LTC Brundage and him prior to Amsterdam that there were no significant findings for the PCR data and both were surprised by the Amsterdam presentation.

On 20 August 92, I took my memo detailing the analysis and results of it to Dr. Vahey's office on Gude Drive. While I was there, COL Burke came in to discuss the memo with us. He asked for additional analyses, in particular, of the data from the 15 patients that were presented in Amsterdam. He had been told by LTC Redfield that they were the first 15 sequential patients that entered the study. I completed the analysis on the first 15 sequential patients that entered the study who had PCR copy data and gave a memo stating how the analysis was done and the results of the analysis to Dr. Vahey on 21 August 92.

Approximately during the time-frame of 21-28 August 92, I had a telephone conversation with COL Burke and LTC Brundage. We discussed the issue of LTC Brundage analyzing the PCR data and I did not object. LTC Brundage came to my office to pick up the PCR data sets and we talked about the PCR analyses and I gave him a copy of my 20 August 92 memo. We discussed the fact that some of the gp160 Phase I patients were taking AZT. It appeared to me that LTC Brundage did not know that some of the gp160 Phase I patients were on AZT. LTC Brundage told me that he did not analyze the data for the Amsterdam presentation and his impression was that there was no significant finding with respect to the viral burden analysis prior to Amsterdam.

During approximately the same time-frame COL Burke came to my office to review all the analyses that I had done for the gp160 Phase I trial; I also showed him the gp120 Phase I trial analyses as well. We talked about the CD4 count stabilization issue and I showed him the longitudinal CD4 count plots per patient that I generate monthly for LTC Redfield. [The same set of plots were shown to COL Dangerfield; I gave the set to COL Dangerfield.] I also showed him the CD4 count longitudinal profiles superimposed and he noted that the CD4 count profiles were, on average, slightly better than what would be expected for natural history patients. [It appeared to me, that he did not know, until I showed him, that, on average, the CD4 count longitudinal profiles of the gp160 Phase I patients were not stabilizing. We also discussed the fact that some of the gp160 Phase I patients were taking AZT. It appeared to me that COL Burke did not know that some of the gp160 Phase I patients were on AZT.]

On 28 August 92, there was a meeting in COL Burke's office to discuss what should be presented and said at the Chantilly 5th Annual Meeting of the National Cooperative Vaccine Development Group for AIDS. Present at the meeting were: COL Burke, LTCs Redfield and Brundage, Dr. Vahey and I. At this meeting, LTC Brundage presented his analysis of the PCR data which validated the findings of my 20 August 92 memo. I presented my 28 August 92 memo which dealt with the additional analyses that COL Burke had asked for on 20 August 92. With respect to LTC Redfield's method of analysis which was used for the Amsterdam viral burden presentation, a consensus was reached at the meeting on the following points: 1.) CD4 adjustment of PCR data was not appropriate, 2.) use of a half log criterion was not appropriate, 3.) the comparison group used was not appropriate, 4.) the statements made by LTC Redfield in Amsterdam regarding viral burden were not correct, and 5.) all the patients should be used in the analysis. LTC Redfield admitted that his analysis in Amsterdam was not appropriate.

There was a meeting of all ranking MMCARR members at the Jackson Foundation main office, 1401 Rockville Pike, on 15 September 92. At this time, LTC Redfield again admitted that his statistical analyses and interpretation of PCR data at Amsterdam were inappropriate and that the analyses performed by LTC Brundage and I were correct. LTC Redfield asked me at the end of this meeting to attend with him that same evening a meeting of the NIH AIDS Vaccine Evaluation Group (AVEG) to address their concern that the increases in viral PCR copies, shown on Dr. Vahey's poster at Chantilly, may be caused by the gp160 vaccine. I explained to Dr. Patricia Fast and the group that there appeared to be no deterministic "post-immunization effect." The "post-immunization effect", assuming that there was no lag effect, appeared to be random. [The data provided to me for analysis would not allow one to determine if there was a lag effect.] However, I also stated that safety concerns with respect to increases in viral PCR copies could never be addressed based on the data that was collected.

During the week of 16 October 92, I was called by the press and an outside individual and told that my 20 August 92 memo was in their possession. I do not know how these people received the memo. I am not very pleased with the fact that someone released my memo to individuals outside the MMCARR. There is no way to tell who leaked the memo because it was widely disseminated internally. I told Mr. Lowe about the calls and he notified COL Burke.

That afternoon LTCs Redfield and Birx, Drs. Vahey and Wohlhieter, Mr. Lowe and Mr. Peterson, and I met with COL Burke in his office to discuss what should be said to the press. LTC Redfield stated that the findings presented in my 20 August 92 memo would be the ones he would present. LTC Birx was upset that I had confirmed to the press that the memo was authentic. Later in this meeting she noted that the 15 patients presented by LTC Redfield at Amsterdam were not the first 15 sequential patients. Also during

this meeting LTC Redfield stated that he never said in Amsterdam that the viral burden went down, that he was misquoted by the press. It should be noted that LTC Redfield also made similar statements prior to and after this meeting. [I had mentioned during this meeting that the member of the press who called me stated that LTC Redfield stated at Amsterdam that the virus was reduced in his gp160 Phase I patients.] Subsequently, in November, the CBS Evening News showed a video tape of LTC Redfield's presentation at Amsterdam and his comments after the presentation. On the video tape LTC Redfield stated that the virus goes down,..that it is significant, reproducible, etc. [I showed a VHS copy of the CBS Evening News segment to COL Dangerfield and gave him a copy of it.] It should also be noted that LTC Redfield showed during his Amsterdam presentation a slide showing the results of his 1/2 log change analysis (his viral burden analysis). On this slide he presented p-values which indicated that the portrayed reduction in viral PCR copies was statistically significant [I gave a copy of this slide to COL Dangerfield].

At this point I would like to discuss some of my concerns with how the gp160 Phase I CD4 count and viral burden data are presented and/or are analyzed.

The problems with LTC Redfield's presentation of CD4 counts are: 1.) Instead of using the actual data, moving averages of five or seven are used. Moving averages do not represent the reality of the CD4 counts [This became evident when the original preliminary versions of the NEJM Figure 4 were re-created by my department. Moving averages of three, five, and seven were used to develop three versions of Figure 4: one version based on the use of moving averages of three, one version based on moving averages of five, and one version based on moving averages of seven. Each version generated a different set of percent change in mean CD4 count curves, with the version generated by the moving averages of seven giving the most dramatic separation in the responders and non-responders curves. I was told that prior to submitting the manuscript to NEJM, LTC Redfield reviewed the original preliminary versions and chose the moving averages of seven version. This was the version published in the NEJM].; 2.) Summarizing data at each time point (e.g., percent change in mean CD4 count) for each group [responders versus non-responders] and connecting these summarized data points to give the impression of a longitudinal profile (an example of this is Figure 4 in the NEJM article) [Note: It is the opinion of my department that this sort of presentation of CD4 counts is inappropriate because it really does not consider the true longitudinal CD4 count profile of each individual patient; one can summarize these individual longitudinal profiles for statistical comparisons between groups (e.g., responders versus non-responders) by using the methods suggested by Laird and Wang, 1990; Dawson and Lagakos, 1991; and Ng, T-H, 1991. Using this approach my department determined that there was no statistical significant difference between the responders and the non-responders CD4 count longitudinal profiles (using the actual CD4 count data that was used to generate the various versions of NEJM

Figure 4]; 3.) Changing category (group) definitions from one presentation to another (e.g., responders versus non-responders (Figure 4 1991 NEJM) and humoral responders versus humoral non-responders (Figure 5, 1992 AIDS Research and Human Retroviruses); 4.) Showing some rather than all patients; 5) The inappropriate use of the term stabilization (i.e., saying that the CD4 counts are stabilizing when they are statistically declining); and 6.) The fact that there was no mention that some patients were taking AZT.

There are also problems with LTC Redfield's Amsterdam viral burden presentation: 1.) Only data from some patients instead of all were presented [A memo written by Dr. Vahey which was shown to me indicated that all 26 patients had available PCR copy data prior to Amsterdam]; 2.) The first 15 sequential patients were not the ones presented. [This became evident when I tried to reproduce (using the same procedures that LTC Redfield said he used) the 1/2 log change analysis that LTC Redfield presented in Amsterdam (The slide of which I showed and gave to COL Dangerfield). This is the analysis that LTC Redfield used to indicate that the viral load (burden) was lowered in his gp160 Phase I patients. I could not reproduce the numbers presented on this slide when I used the first sequential 15 patients who had PCR data. I called Dr. Vahey and mentioned to her that the first sequential 15 patients could not have been used in LTC Redfield's 1/2 log change analysis; that the data for these patients could not generate the numbers on his Amsterdam presentation slide. Dr. Vahey asked Dr. Birx about this issue and Dr. Birx told Dr. Vahey that LTC Redfield told her (Birx) that the first sequential 15 patients were not used in the Amsterdam presentation.]; 3.) An appropriate comparison group was not used in his 1/2 log change analysis [The natural history historic controls were not comparable to the gp160 Phase I patients in terms of CD4 counts, WR stages, etc. I was told by several infectious disease physicians that the patients in the natural history historic control (comparison) group were sicker (i.e., had been infected longer and/or had lower CD4 counts); 4.) LTC Redfield used CD4 adjustments on the viral PCR copy data [It was affirmed at the 28 August 92 meeting that this procedure artificially accentuated the viral burden differences between the gp160 Phase I patients and the patients in the natural history historic control group.]; 5.) The use of the 1/2 log change criterion for the demonstration of a meaningful change in PCR copy. [Without knowing what the biological and clinical significance is when viral PCR copies change by various factors (e.g., 1/2 log), it is inappropriate to categorize patients based on this 1/2 log criterion. This determination was affirmed at the 28 August 92 meeting.]; 6.) The use of inappropriate baseline values for PCR copy data. [LTC Redfield include post-immunization PCR values (i.e., PCR copy data which measured viral load after the patient was vaccinated) in his baseline. Therefore, a true pre-vaccination baseline was not used in LTC Redfield's analysis. It was noted by some individuals that this type of baseline (the one LTC Redfield used) could actually create an elevated baseline and in conjunction with the CD4 adjustment procedure make it appear that there was a

decrease in viral PCR copy when in reality there was no such reduction or if there was a reduction, not one as dramatic.]; 7.) There was no mention that some of the gp160 Phase I patients were on AZT [In any circumstance this should be mentioned because it is an indication of the health status for the gp160 Phase I patients. In addition, if any of the 15 patients presented in Amsterdam were taking AZT and any of their PCR copy data were generated during AZT use, this would create problems with determining whether the effect was caused by gp160 or by AZT: I have been told by infectious disease physicians that AZT does reduce viral load.]; and 8.) In his presentations subsequent to Amsterdam, LTC Redfield states that viral load increases in the natural history group but not in his gp160 Phase I patients. My 20 August 92 memo shows that this is not correct - there was no significant difference between the two groups (gp160 patients versus the natural history historic control patients) in terms of change in PCR copy from baseline (using both LTC Redfield's baseline and the baseline Dr. Vahey preferred (Dr. Vahey's baseline contained only pre-vaccination PCR copy data - thus two separate analyses were done, one for each type of baseline). As mentioned early, it was determined that the patients in the natural history historic control group were not comparable. Infectious disease physicians told me that this lack of comparability gave a worst case scenario for the natural history historic control group because they were infected longer and/or had lower CD4 counts and therefore would in all likelihood have higher viral load than the gp160 Phase I patients. Even with this worst case scenario my 20 August 92 memo results indicated no significant difference between the two groups. In addition, there was no significant difference between baseline and endpoint for either group in terms of PCR copies. (Again, using both definitions for baseline - two separate analyses were done). When LTC Redfield stated that it is known that viral burden increases in a natural history cohort but does not in the gp160 Phase I patients, this is also a misleading statement. Comparing a natural history group of patients who have been infected longer to the gp160 Phase I patients who have been infected for a shorter amount of time is not appropriate, according to the various infectious disease physicians that I have talked with. From a statistical point of view, in order to have a meaningful comparison, one would need to compare two groups that had been infected for approximately the same amount of time (i.e., compare two groups with early HIV infection).

It is my belief that when people are allowed to make presentations without careful coordination with statisticians there are inevitable problems and this needs to be addressed. For the integrity of the MMCARR there needs to be an internal review process to review and clear presentations and publications. In my opinion, if LTC Redfield had made it clear at Chantilly what the real situation was regarding the longitudinal CD4 count profiles of the gp160 Phase I patients, what the real situation was regarding the viral PCR copy data of the gp160 Phase I patients and that some of these patients are taking AZT, this whole issue would have been finally settled. In addition, some of these problems are caused by

the misuse of terms (e.g., stabilization of CD4 counts). This can be viewed as a semantic problem, but if it misleads the public and the scientific community with respect to the findings of the gp160 Phase I trial, it becomes a serious scientific problem.

Recently I have received calls from the press regarding comments from individuals close to LTC Redfield. These individuals have told the press (Nancy Tomich, U.S. Medicine) a number of misleading and false statements concerning my analyses of the viral burden data. In addition, these sources told Nancy Tomich that I was presenting misinformation regarding the PCR copy data analyses. I have been and am a MMCARR team player. I have not at any time presented to MMCARR or the press any misinformation regarding the PCR copy data analyses.

Finally, with respect to the Amsterdam viral burden presentation, I believe it is in the best interest of MMCARR that LTC Redfield indicates: 1.) Which 19 natural history patients were analyzed and why they were chosen; 2.) Which 15 gp160 Phase I patients he actually analyzed and why they were chosen; 3.) Whether any of these 15 gp160 Phase I patients were on AZT; 4.) How he generated the numbers (percentage of patients falling into the categories: 1/2 log increase; 1/2 log decrease or no change; and 1/2 log decrease) recorded on the "Alteration of HIV Specific DNA and RNA" slide he presented (his 1/2 log change analysis); 5.) How he has determined that the longitudinal CD4 count profiles of the gp160 Phase I patients have stabilized; and 6.) Why he has failed to mention that some of the gp160 Phase I patients are taking AZT.

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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. Response was associated with the CD4 cell count determined before vacci-

nation (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 (67 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.¹⁻⁶ 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.¹⁴⁻²⁰ 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 infection²¹⁻²³; some investigators have deduced that this is a consequence of viral-directed events such as changes

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The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

in viral regulatory proteins or changes in viral cytopathogenicity.^{24,25}

An alternative hypothesis is that both the prolonged clinical course of HIV infection and the progressive increase in the expression of HIV in vivo in the late stages of disease may be direct consequences of the effectiveness of the immune response to HIV in its early stages.²⁶⁻²⁸ In short, as the antiviral immunity gradually weakens, poor control of in vivo viral replication results.²⁹ To test this hypothesis, we explored the possibility of augmenting HIV-specific immunity in infected persons by active immunization with an HIV-protein product, gp160. This product has recently been shown to be safe and immunogenic in healthy adults without HIV infection.²⁹ The objective of the present phase I trial was to evaluate the safety and immunogenicity of active immunization with recombinant gp160 in volunteers with early HIV infection and to determine the feasibility of using this intervention to modify the immune response to HIV in subjects with chronic infection.

METHODS

A more detailed description of our methods is available from the National Auxiliary Publications Service.*

Selection of Subjects

Thirty volunteer subjects with HIV infection were recruited from among Department of Defense health care beneficiaries. The nature of the trial was explained in detail to each subject, and written informed consent was obtained. Only seropositive patients in an early stage of HIV infection, defined as Walter Reed stage 1 or 2 (a CD4 cell count of not less than 400 per milliliter for more than three months, with or without lymphadenopathy),¹³ were eligible for enrollment. The subjects also had to be between 18 and 50 years old, have a normal complete blood count, have no evidence of end-organ disease, have not abused alcohol or drugs over the preceding 12 months, and have not received antiretroviral or immunomodulatory drugs. All the subjects underwent a two-month base-line evaluation before randomization. None received any antiretroviral or immunomodulatory drug during the trial.

Vaccine Product and Immunization Schedule

The test vaccine was a noninfectious subunit glycoprotein derived from human T-cell lymphotropic virus Type III, gp160 (VaxSyn HIV-1, MicroGeneSys, Meriden, Conn.), a baculovirus-expressed recombinant protein produced in the cells of lepidopteran insects, biochemically purified, and adsorbed to aluminum phosphate for final formulation.²⁹ Three doses of gp160 were used: 40, 160, and 640 μ g. Both the 40- μ g and 160- μ g doses were injected in a volume of 1 ml; the 640- μ g dose was given as 320 μ g per milliliter in a volume of 2 ml.

The 30 subjects were assigned to six vaccination groups of 5 subjects each. Two immunization schedules were investigated: schedule A, with vaccination on days 0, 30, and 120, and schedule B, with vaccination on days 0, 30, 60, 120, 150, and 180. Three of the six groups received different doses of vaccine according to schedule A, and the other three groups received different doses according to schedule B (Table 1). All vaccinations were administered by intramuscular injection into the deltoid muscle. The

Table 1. Immunization Schedule.

SCHEDULE AND GROUP NO.	STUDY DAY					
	0	30	60	120	150	180
	<i>μg of gp160/ml</i>					
Schedule A						
1	40	40	—	40	—	—
3	160	160	—	160	—	—
5	640	640	—	640	—	—
Schedule B						
2	40	40	40	160	160	160
4	160	160	160	640	640	640
6	640	640	640	640	640	640

duration of the trial was 10 months — i.e., a 2-month base-line evaluation and an 8-month follow-up evaluation after the initial vaccination.

Assessment of Safety

Each subject was interviewed and examined on days 0, 1, 2, 3, 15, and 30 after each injection. They were asked whether they had had fever, chills, nausea, vomiting, arthralgia, myalgia, malaise, urticaria, wheezing, dizziness, or headache and were examined for local reactions at the site of injection, including erythema, swelling, itching, pain and tenderness, skin discoloration, skin breakdown, any change in regional lymphadenopathy, any change in the function of the extremity into which the vaccine had been injected, and the formation of any subcutaneous nodules at the site of injection. The complete blood count, serum biochemical determinations, coagulation-profile assessment, and urinalysis were performed monthly.

In vitro cellular immune function was assessed by determining the T-cell phenotype (total-lymphocyte, CD4 cell, and CD8 cell phenotypes)^{30,31} and the T-cell proliferative response to mitogens (pokeweed and concanavalin A) and control antigens (*Candida albicans* and tetanus).³¹ In vivo cellular immune function was assessed by skin testing for delayed hypersensitivity to control antigens (mumps, tetanus toxoid, *C. albicans*, and trichophyton).

Quantitative viral culture of peripheral-blood mononuclear cells and plasma,²² DNA polymerase-chain-reaction testing,³² and measurement of serum p24 antigen levels were performed to monitor the HIV viral load in vivo.

Assessment of Immunogenicity

Antibodies directed against whole HIV proteins were measured with both recombinant viral gene products gp160, p66, and p24 (MicroGeneSys) and whole viral lysate of prototype HIV strain MN by dot blotting and Western blotting techniques.³³ Antibody responses to specific envelope epitopes were also measured (Table 2).

Neutralization activity was measured against three prototype HIV isolates (HIB, RF, and MN) in a syncytium inhibition assay.³⁷ HIV-specific cellular responses were measured by standard lymphocyte-proliferation-assay techniques with use of gp160, p24, and baculovirus-expression-system control protein.³¹ A detailed description of the methods for assessing safety and immunogenicity is available elsewhere.*

Definition of Response

The subjects were classified as responding to vaccination if they had a reproducible selective increase in both a cellular and a humoral immune response against HIV envelope-specific epitopes that was temporally associated with the series of vaccinations. Vaccine-induced humoral immunity was indicated by seroconversion to HIV envelope-specific epitopes, a secondary booster immune response to envelope-specific epitopes, or both. Vaccine-induced cellular immunity was indicated by the development of a new, reproducible, temporally associated proliferative response to gp160.

Subjects with neither a humoral nor a cellular proliferative response, or only a humoral or only a cellular proliferative response,

*See NAPS document no. 04868 for eight pages of supplementary material. Order from NAPS c/o Microfilm Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163-3513. Remit in advance (in U.S. funds only) \$7.75 for photocopies or \$4 for microfiche. Outside the U.S. and Canada add postage of \$4.50 (\$1.50 for microfiche postage). There is an invoicing charge of \$15 on orders not prepaid. This charge includes purchase order.

Table 2. Humoral Response (Antibody to HIV Envelope Epitope) and Cellular Response (T-Cell Proliferation) to Vaccination.*

	PATIENT NO.	GROUP NO.	HUMORAL RESPONSE — ANTIBODY TO SPECIFIC EPITOPES											CELLULAR RESPONSE		
			49	88	106	241	254	300	308	342	422	448C	582	735	TOTAL gp160	PEAK LSI
Responders																
Schedule A	8	1	+	+	+	-	-	⊕	⊕	⊕	⊕	⊕	+	+	⊕	25
	15	1	⊕	+	+	-	⊕	+	+	-	-	+	+	-	⊕	71
	17	1	⊕	-	+	-	-	+	+	-	-	+	+	+	⊕	23
	19	5	⊕	-	⊕	⊕	-	⊕	⊕	⊕	-	⊕	+	-	⊕	50
	20	5	-	HB	⊕	-	-	-	+	+	-	⊕	+	-	⊕	19
	22	5	+	-	+	-	-	-	-	-	-	+	-	-	⊕	16
Schedule B	1	2	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	⊕	+	-	⊕	102
	3	2	⊕	⊕	⊕	-	-	-	-	-	-	+	+	⊕	⊕	171
	16	2	⊕	⊕	⊕	-	-	-	⊕	⊕	⊕	-	+	+	⊕	41
	19	2	⊕	⊕	⊕	-	-	-	⊕	⊕	⊕	-	+	+	⊕	20
	6	4	⊕	⊕	⊕	-	-	-	-	-	-	-	+	+	⊕	34
	7	4	⊕	⊕	⊕	-	-	-	⊕	⊕	⊕	-	+	+	⊕	95
	13	4	+	+	+	-	-	-	+	+	-	-	+	+	⊕	27
	28	4	-	⊕	⊕	-	-	-	⊕	ND	-	-	+	+	⊕	3
	32	4	⊕	⊕	⊕	-	-	-	⊕	⊕	⊕	-	+	+	⊕	35
	11	6	+	⊕	⊕	-	-	-	⊕	⊕	⊕	-	+	+	⊕	24
	23	6	⊕	⊕	⊕	-	-	-	⊕	⊕	⊕	-	+	+	⊕	8
	29	6	⊕	⊕	⊕	HB	-	-	⊕	⊕	⊕	-	+	+	⊕	158
	33	6	⊕	-	⊕	-	-	-	⊕	⊕	⊕	-	+	+	⊕	28
Nonresponders																
Schedule A	2	1	-	-	+	-	-	+	+	-	-	+	+	-	.	
	18	1	-	+	-	-	-	-	-	-	-	-	+	+	.	
	5	3	+	-	-	-	-	-	+	+	-	-	+	+	.	
	12	3	+	+	+	-	-	-	-	-	-	-	+	+	.	
	14	3	+	-	+	-	-	-	+	+	-	-	+	+	.	
	21	3	-	-	+	HB	-	+	HB	-	-	-	+	+	.	
	35	3	-	-	⊕	-	-	-	-	-	+	+	+	-	.	
	27	5	-	-	+	-	-	-	ND	-	-	-	+	-	⊕	10
	31	5	+	-	-	-	-	+	+	-	-	-	+	+	.	
	24	6	-	-	⊕	-	-	+	+	-	-	-	+	-	⊕	28

*Epitopes 88 (amino acids 88 to 98 in gp120) and 448C (amino acids 448 to 514 in gp120) were selected because antibody directed against these regions of gp120 is reported to correlate with early-stage HIV infection.^{33,36} Epitopes 106 (amino acids 106 to 121 in gp120), 241 (amino acids 241 to 272), 254 (amino acids 254 to 272), 300 (amino acids 300 to 340), 308 (amino acids 308 to 322), 422 (amino acids 422 to 454), and 735 (amino acids 735 to 752) were selected because of their putative functional importance (106 and 422 have been implicated in CD4 binding^{33,36}; 241, 254, and 735 have been implicated in group-specific neutralization^{33,36}; and 300 and 308 have been implicated in type-specific neutralization^{33,36}). Epitope 582 (amino acids 582 to 602) was selected as a control because it represents the immunodominant envelope domain in natural HIV infection.^{33,43,46} Additional epitopes investigated included 49 (amino acids 49 to 128) and 342 (amino acids 342 to 405).

A circle around a symbol denotes a documented change in the HIV-envelope-directed immune response. A circled minus sign denotes a primary humoral response, and a circled plus sign a secondary humoral response; a minus sign without a circle denotes an antibody negative to specific epitope before and after immunization, and a plus sign without a circle an antibody positive to specific epitope before and after immunization, but no quantitative change. A circled dot denotes a new T-cell proliferative response to gp160 after immunization, and a dot without a circle the lack of a cellular response to gp160. HB denotes high background (result not interpretable), and ND not done.

to gp160 epitopes or HIV envelope epitopes were classified as not having responded to the vaccination.

Statistical Analysis

Proportions were compared by Fisher's exact test (two-sided). Changes in cellular immune responses were summarized as the magnitude of changes (fold change) in the lymphocyte-stimulation index. The fold change for each subject was calculated by dividing the mean of the values for the index that were measured after the last vaccination by the mean of the values for the index at base line. Differences between subgroups in cellular immune responses were assessed by comparing the distributions of fold changes by the Wilcoxon rank-sum test. Changes in the number of CD4⁺ T lymphocytes were compared between subgroups of subjects and with the change expected on the basis of experience with the natural history of HIV infection. Comparisons between subgroups were based on the mean of the percent changes in CD4 cell counts at the end of the follow-up period, as compared with the means at base line. At each time point, the number of CD4⁺ T lymphocytes was calculated as the mean of seven values (the median was determined according to the time point).

RESULTS

Demographic and Base-Line Clinical Characteristics

Twenty-six of the 30 subjects were men, and 4 were women. Fourteen were non-Hispanic whites, 13 were black, and 3 were Hispanic. Their mean age was 29

years (range, 18 to 49). At enrollment 8 subjects had HIV infection in Walter Reed stage 1, and 22 had infection in stage 2.³⁷ The base-line mean CD4 cell count was 668 per milliliter (range, 388 to 1639). The mean time between initial diagnosis and study entry was 24 months (range, 3 to 49).

Vaccine-Induced Humoral Responses

All 30 subjects completed the 240-day trial. Nineteen (63 percent) had a vaccine-induced augmentation of both HIV gp160-specific humoral and cellular immune responses and thus were classified as "vaccine responders." Of the 11 subjects classified as "nonresponders," 4 had only a humoral or a cellular immune response and 7 had no detectable response; all 7 without a response had received only three doses of vaccine (schedule A). No subject had changes in antibody binding to HIV polymerase (p66) or structural (p24) gene products or to the non-HIV control antigen tetanus. No antibody to the baculoviral lepidopteran-cell control protein developed in any subject.

Increases in the level of envelope antibody (gp160) were detected in 13 subjects on Western blotting with

the whole-virus lysate HIV-MN. These changes were related to the immunization schedule. Three of 15 subjects (20 percent) assigned to schedule A and 10 of 15 (67 percent) assigned to schedule B had an increase in the level of antibody to envelope proteins ($P = 0.025$ by Fisher's exact test, two-tailed). All 13 subjects also seroconverted to specific envelope epitopes. Conversely, of the 10 subjects who did not seroconvert to any envelope-specific epitope, none had an increase in envelope-antibody levels on Western blotting. The remaining seven subjects who seroconverted to specific envelope epitopes had no change in whole-virus envelope antibody on Western blotting. No changes in antibody directed against non-HIV envelope proteins were observed in any subject.

Fourteen of 15 subjects (93 percent) assigned to schedule B (six doses) had an increase in total gp160 antibody, as opposed to only 7 of 15 (47 percent) assigned to schedule A (three doses) ($P = 0.01$ by Fisher's exact test, two-tailed) (Table 2). The range of the prevalence of 11 of the 12 gp160-specific epitopes selected for study (Table 2), from before to after vaccination, was as follows: epitope 49, 27 to 70 percent; epitope 88, 28 to 52 percent; epitope 106, 50 to 87 percent; epitope 241, 0 to 14 percent; epitope 254, 0 to 13 percent; epitope 300, 47 to 77 percent; epitope 308, 42 to 69 percent; epitope 342, 0 to 27 percent; epitope 422, 3 to 10 percent; epitope 448C, 73 to 87 percent; and epitope 735, 17 to 33 percent (Fig. 1). Vaccine-induced seroconversion was noted to all the specific epitopes, except epitope 582 (Table 2). Antibodies (seroconversion) directed against epitopes 241, 254, and 342 were detected only after vaccination (Table 2).

Secondary immune responses to epitopes 88, 106, 300, 308, 448C, and 582 were elicited (Table 2). The prevalence of antibody directed against epitope 582 was 100 percent before vaccination, and only one subject (3 percent) had a secondary immune response.

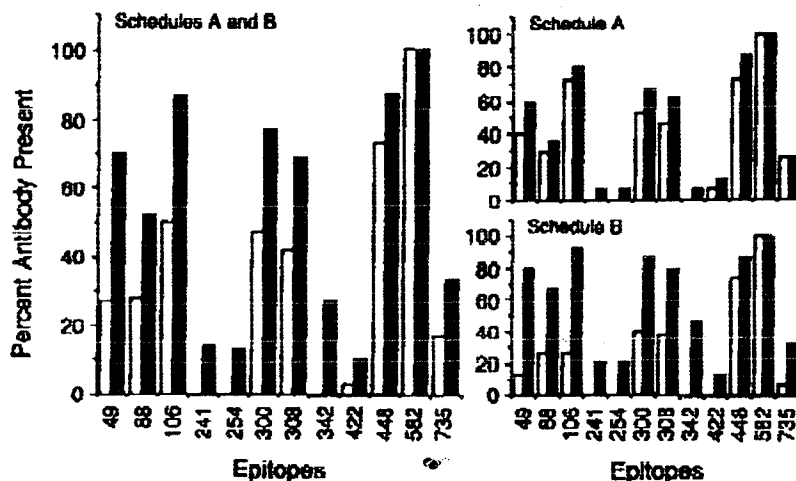


Figure 1. Prevalence of Vaccine-Induced Antibody Directed against Specific HIV Envelope Epitopes, before Immunization (Open Bars) and after Immunization (Solid Bars, Study Day 195 to 240), According to Immunization Schedule.

Three injections were given during schedule A, and six during schedule B.

The pattern of vaccine-induced HIV antibody to envelope epitopes was variable (Table 2). Primary antibody responses (seroconversion) to at least one epitope occurred in 20 subjects — 14 of 15 assigned to schedule B and 6 of 15 assigned to schedule A ($P = 0.005$ by Fisher's exact test, two-tailed). Furthermore, of all the epitopes studied, subjects assigned to schedule A seroconverted to only 15 of 110 (14 percent) of the potential epitopes to which they had no antibodies before vaccination, whereas subjects assigned to schedule B seroconverted to 60 of 129 potential epitopes (47 percent) ($P < 0.0001$ by Fisher's exact test, two-tailed). Seroconversion to three or more envelope epitopes occurred in 9 subjects (60 percent) assigned to schedule B but in only 2 (13 percent) of those assigned to schedule A ($P = 0.02$ by Fisher's exact test, two-tailed).

Serum neutralization activity against three distinct strains (HIV-IIIB, HIV-MN, and HIV-RF) was determined on days 0, 90, and 195 in seven subjects. Four of five responders had increasing neutralizing activity to one or more isolates, as compared with neither of two nonresponders. Furthermore, the responders as a group, unlike the nonresponders, had an increase in the percentage of inhibition at a given dilution of serum required to inhibit syncytium formation against each prototype isolate tested.

Vaccine-Induced Cellular Responses

In 21 of 30 subjects (70 percent), a new T-cell proliferative response to gp160 developed after vaccination (Table 2). Figure 2 shows the time course of proliferative responses to gp160, p24, and a baculovirus control protein in four typical vaccine responders. In all subjects, the gp160-induced proliferation increased, in that the mean lymphocyte-stimulation index rose from 3 at base line to 10 (a value calculated from the mean of four values determined after the last immunization). In contrast, no

change was noted in the proliferative responses directed against HIV p24 protein or the control baculovirus protein. Vaccine-induced changes in the mean lymphocyte-stimulation index for all subjects, for subjects grouped according to degree of response, and for subjects grouped according to immunization schedule are shown in Figure 3. The change in proliferative response to gp160 in the vaccine responders was significantly different from that in the nonresponders ($P < 0.001$ by Wilcoxon test, one-tailed). The proliferative responses induced by the six injections of gp160 according to schedule B were greater than those induced by the three injections according to schedule A (Fig. 3) ($P < 0.10$ by Wilcoxon test, one-tailed).

Nineteen of the 21 subjects who

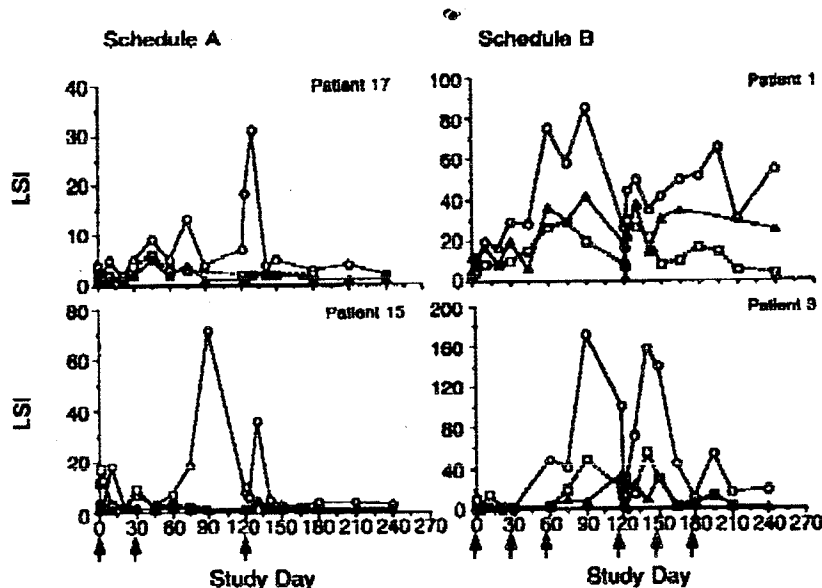


Figure 2. Vaccine-Induced T-Cell Proliferation of gp160 in Four Responders, as Reflected by the Lymphocyte-Stimulation Index (LSI).

Arrows denote the days on which immunizations were administered. The LSI responses to gp160 are represented by open circles, those to p24 by open squares, and those to the expression-system baculoviral control protein by solid triangles. Note the temporal relation between vaccination and the increase in gp160 proliferative response.

had proliferative responses to gp160 also had a humoral response (the 19 responders). The maximal mean lymphocyte-stimulation index observed among all 19 responders in response to gp160 was 50.1. However, in each responder the index was variable (range of peak values, 3 to 171) (Table 2), as was the temporal relation between vaccination and the magnitude and duration of the cellular responses to gp160 (Fig. 2).

Predictors of Immune Responsiveness

Despite the limited size of the sample in this trial, several factors were demonstrated to be associated with vaccine-induced immunogenicity. Six of 15 (40 percent) of the subjects assigned to schedule A responded, as compared with 13 of 15 (87 percent) of those assigned to schedule B ($P = 0.02$ by Fisher's exact test, two-tailed) (Table 2). Of the 16 subjects with a mean base-line CD4 count greater than 600 per milliliter, 13 (81 percent) were responders, as opposed to 6 of 14 (43 percent) whose mean CD4 count at entry was 600 or fewer cells per milliliter ($P = 0.07$ by Fisher's exact test, two-tailed). Multiple immunizations improved immunogenicity, even among patients with base-line CD4 counts of 600 or fewer cells per milliliter; five of six subjects with such counts assigned to schedule B (six injections) were responders, as compared with only one of eight assigned to schedule A (three injections) ($P = 0.03$ by Fisher's exact test, two-tailed; Table 3).

Toxicity

No evidence of systemic toxicity was observed, but local reactions were noted in 87 percent of the subjects

(13 in each vaccination group). These reactions included induration, tenderness, and transient subcutaneous nodule formation at the injection site; an increase in regional adenopathy was rarely noted. No subject refused a booster injection. No difference in the frequency of local reactions was observed in relation to primary immunization, booster injection, or vaccine dosage.

No evidence of an adverse effect on the immune system was demonstrated, as measured in vitro by mitogen-specific and antigen-specific proliferative responses, in vivo by responses to delayed-hypersensitivity skin testing, or by acceleration of quantitative CD4 cell depletion. At base line the mean CD4 cell count was 716 in the responders and 605 in the nonresponders; from study day 180 to day 240 the mean count was 714 and 561, respectively. During the course of the 240-day trial, the net change in the mean CD4 cell count among the responders was a decrease of 0.2 percent, whereas among the nonresponders it was a decrease of 7.3 percent (Fig. 4). Vaccine-induced immunogenicity to HIV was not associated with evidence of an accelerated

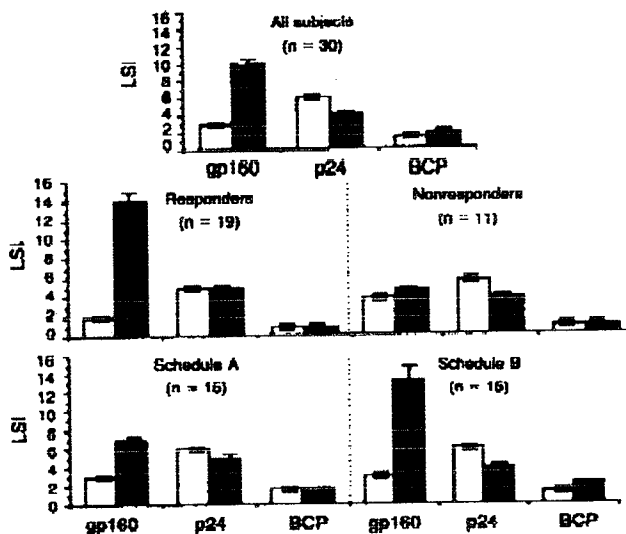


Figure 3. Lymphocyte Proliferative Responses Associated with Vaccination.

Open bars represent the mean (\pm SEM) change in base-line values for the lymphocyte-stimulation index (LSI), and solid bars the mean values of the four sequential LSI values measured after the last immunization in each subject. Values are shown for all subjects combined, subjects grouped according to responsiveness, and subjects grouped according to immunization schedule. The HIV protein sources gp160 and p24 were produced by a lipid-teran baculoviral expression system; BCP represents the expression-system baculoviral control protein.

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)	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

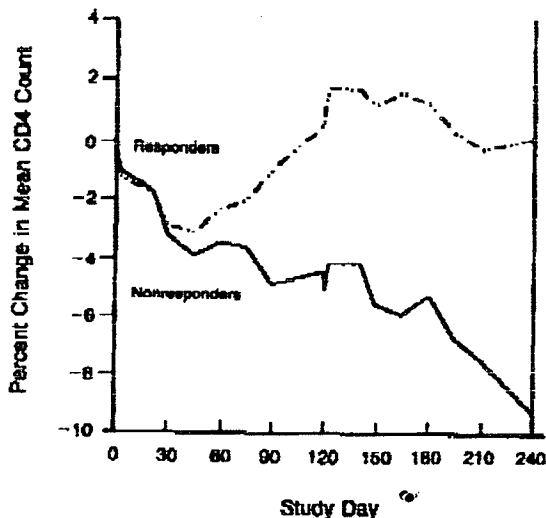


Figure 4. Change from Base Line in the CD4 Cell Count in Responders and Nonresponders.

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).

explored. Although there are other examples of post-infection 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.¹⁸

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 hypo-responsiveness 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.³²

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 cell 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.

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HIV-Specific Vaccine Therapy: Concepts, Status, and Future Directions

ROBERT R. REDFIELD, and DEBORAH L. BIRX

The physician of the future will be an immunisator [sic] A.E.
Wright *Studies on Immunisation* [sic] (1943)

VACCINE THERAPY, A HISTORICAL PERSPECTIVE

THE PRESENT CONCEPT OF VACCINES focuses on the prophylactic role. However, vaccinology at the turn of the century was predominantly a therapeutic field. In the 1900s several prominent clinical scientists, Pasteur, Koch, and Wright, proposed that vaccination with a specific antigen could be utilized as an effective treatment against the disease caused by an organism from which the antigen was derived.^{1,2} In 1910 Sir Almroth Wright (considered the Father of Modern Vaccine Therapy) summarized the state of the art of vaccine therapy at a session of the Royal Society of Medicine entitled: "Vaccine Therapy: Its Administration, Value and Limitations."³ After a considerable series of discussions and debates, Dr. Wright conceded two major limitations: "1) Limitations as contended for by the clinicians who regard vaccine therapy as an uncomfortable innovation. 2) Limitations as contended for by the bacteriological worker who looks forward to vaccine therapy being applied in conformity with scientific principle."³ Although vaccine therapy had been pursued in the treatment of several diseases such as tuberculosis and leprosy, lack of conformity to scientific principle haunted the research: the results were considered speculative and lacking supportive evidence.^{4,5} Thus, as we began to explore the use of HIV-specific vaccine therapy nearly a century later, these same objections remained.

In 1985 Zagury, pioneered the investigation of the application of vaccine therapy in the setting of HIV infection.^{6,7} In 1987, Salk also endorsed this approach.⁸ Significant support was provided by Stanberry and colleagues in 1987 when they demonstrated evidence that postinfection vaccination with recombinant herpes simplex glycoproteins modified the natural history of herpes diseases in a guinea pig model.⁹ These data

provided the first evidence that scientific principles may be consistent with the concept of vaccine therapy.

THE QUESTION: DOES NATURAL INFECTION WITH A PATHOGEN DEFINE THE LIMITS OF MAN'S IMMUNE RESPONSE TO THAT PARTICULAR PATHOGEN?

The consequences of viral host interaction form the basis of the natural history of a pathogen's life cycle and disease potential in humans. The consequences of viral host interaction range from acute viral infection (self-limited, nonrecurrent or self-limited, recurrent) to chronic viral infection (latency with or without transient viral expression, or chronic viral expression resulting in progressive disease).

The human immunodeficiency virus (HIV) in man is a chronic viral infection. Yet natural infection with HIV elicits a vigorous host-directed immune response.¹⁰ Despite this naturally elicited immune response, HIV infection results in a chronic progressive debilitating disease. The host-directed response is not effective in clearing HIV or in controlling its replication to prevent progressive end organ damage. Several possibilities exist to explain this consequence of viral host interaction. It may be that the human host is genetically restricted in its capacity to respond to important HIV-specific regions critical to effective immunoregulation; that the host response to these regions, while present, is suboptimal; or that critical epitopes may be masked preventing immunologic presentation. Conversely, HIV may escape from immunologic control as a consequence of genomic diversity or HIV may not be susceptible to immunologic controls. We hypothesize that man does possess the capacity to generate an effective immunoregulatory response to HIV. However, under natural conditions this response is inadequate.

In 1985 we proposed a model for HIV infection which hypothetically defined a temporal interrelationship between viral load and replication capacity, host directed immune regulation and clinical end organ damage (Fig. 1).¹¹ With this model

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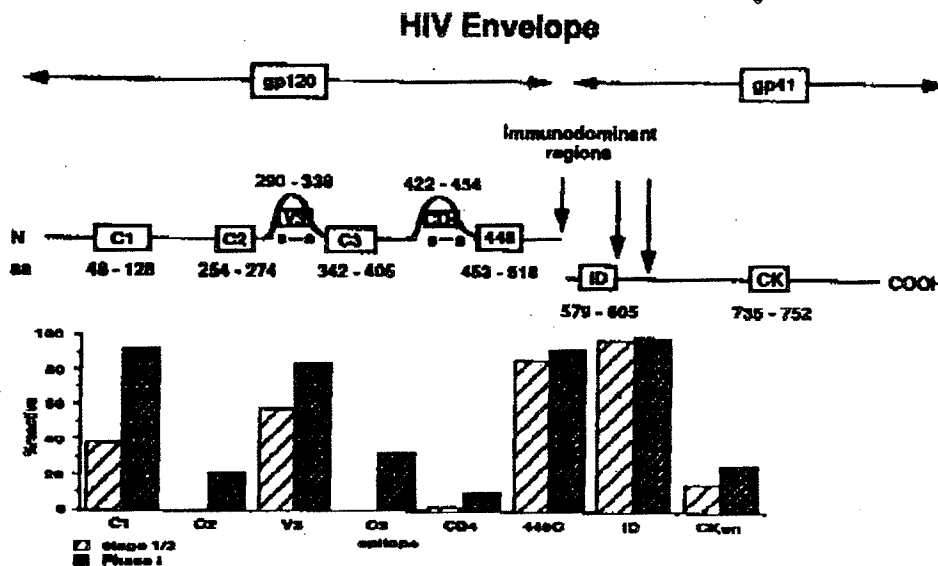


FIG. 3. Antibody reactivity against HIV envelope regions in 177 early stage patients prior to vaccine therapy and 27 Phase I rgp160 (MicroGeneSys) recipients. The boxed regions represent the regions (amino acid sequences below) of malE fusion proteins prepared from gp160 (LAI) (MicroGeneSys). ▨ 354 serum from 177 early stage (WR 1/2) individuals were reacted using standard Western blot techniques and reactivity expressed as a percent. ▩ Represents the antibody profiles from the 27 of the 28 Phase I patients after completion of a six-shot reimmunization series.

therapy to broaden the host-directed anti-HIV envelope immune response and to assess the safety of such a vaccine. Thirty individuals were randomized into six groups varying in dosage (40, 160, and 640 μ g) and schedule (3 or 6 injection regimen).

A major scientific obstacle to acceptance of vaccine therapy was the lack of scientific evidence regarding the feasibility of antigen-specific augmentation/redirection of the host-directed immune response in the setting of any chronic viral infection. For this reason, we developed an arbitrary, yet highly restrictive, case definition of a "vaccine responder." A responder was defined as an individual who developed both a humoral and cellular alteration in immune response, specific for the immunogen gp160, which was reproducible and temporally associated with vaccination. Thus a response was based strictly on the documentation of newly acquired anti-HIV envelope immunological mechanisms, regardless of clinical response.

Of the 30 volunteers, 19 developed both new humoral and cellular anti-HIV envelope-directed immune responses. Table 1 summarizes the seroconversions and secondary immune responses to envelope epitopes and the T-cell anti-envelope proliferative responses elicited postvaccination. Vaccine-induced immune responses included seroconversion to conserved regions of the gp120 molecule C1, C2, and C3; induction of T-cell recognition and proliferation to gp120 and gp160 and the development of cytotoxic T-cell responses to envelope-derived peptides.

At the outset of the trial, there was significant concern regarding the safety of vaccine therapy using HIV envelope-derived products. To address safety issues, patients were enrolled in a staggered fashion. No systemic toxicity was experienced. Local toxicity was anticipated (as it would be with any injection series) and was limited to induration and tenderness. No evidence of immune-specific toxicity was demonstrated as

measured *in vitro* by antigen-specific proliferation responses to tetanus and candida; and *in vivo* by delayed hypersensitivity testing and monitoring quantitative CD4 counts and the rate of CD4 decline.

With the results of this Phase I trial, we documented the feasibility of postinfection vaccination in the setting of early stage HIV infection and provided the scientific evidence for vaccine therapy previously lacking. In addition, we established that natural infection with a pathogen does not define the limits of human immune response to that particular pathogen. Clinical investigators have the ability to modify, expand, and potentially direct the host-directed immune response to a chronic pathogen such as HIV.

CONTINUATION PHASE I rgp160 TRIAL

The Phase I trial was completed in November 1990. Following the completion of the study, a continuation study was initiated. Of the original 30 volunteers, 28 re-enrolled in this continuation trial. Original vaccine responders received 160 μ g every 120 days; and the original nonresponders and poor responders received a 6-injection regimen of 640 μ g on days, 0, 7, 30, 60, 90, and 120, and subsequent booster injections (160 μ g) every 120 days. This trial had several scientific objectives: (1) To assess the duration and ability to boost vaccine induced anti-envelope-directed cellular and humoral responses; (2) to further define the limits of vaccine therapy to broaden host-directed anti HIV envelope responses; (3) to determine why vaccine nonresponders failed to demonstrate vaccine immunogenicity and determine if additional immunizations would be of value; (4) to assess the long-term safety of this product in patients with HIV infection.

TABLE 1. SUMMARY gp160 VACCINE-INDUCED IMMUNE RESPONSES INITIAL PHASE I TRIAL

	Patient	* Humoral antibody response to specific envelopes						Cellular response T-cell proliferation	
		49	241	300	342	448	582	735	Total gp160 (peak LSI)
3-INJECTIONS	8	+	-	-	-	+	+	+	• (25)
	15	-	-	+	-	+	+	-	• (71)
	17	-	-	+	-	+	+	+	• (23)
	10	-	-	-	-	-	+	-	• (50)
	20	-	-	-	-	-	+	-	• (19)
	22	+	-	-	-	+	+	-	• (16)
	2	-	-	+	-	+	+	-	•
	18	-	-	-	-	-	+	-	•
	5	+	-	+	-	+	+	+	•
	12	+	-	-	-	+	+	+	•
	14	+	-	+	-	+	+	+	•
	21	-	-	+	-	+	+	-	•
	35	-	-	-	-	+	+	-	•
	27	-	-	+	-	-	+	-	•
	31	+	-	-	-	+	+	-	• (10)
6-INJECTIONS	1	-	-	-	-	+	+	-	•
	3	-	-	-	-	+	+	-	• (102)
	16	-	-	-	-	+	+	-	• (171)
	19	-	-	-	-	+	+	-	• (41)
	6	-	-	-	-	+	+	-	• (20)
	7	-	-	-	-	+	+	-	• (34)
	13	+	-	+	-	+	+	-	• (95)
	28	-	-	+	-	-	+	-	• (27)
	32	-	-	+	-	+	+	-	• (3)
	11	+	•	-	-	+	+	-	• (35)
	23	-	-	-	-	+	+	+	• (24)
	29	-	-	-	-	+	+	-	• (8)
	33	-	-	+	-	+	+	-	• (158)
	4	-	-	+	-	+	+	-	• (28)
	24	-	-	+	-	-	+	-	• (28)

Epitope 49 (AA 40-123 in gp120), 241 (AA 254-274 in gp120), 300 (AA 300-340 in gp120), 342 (AA 342-405 in gp120), 448C (AA 453-518 in gp120), 582 (AA 579-605 in gp41), and 735 (AA 735-752 in gp41). around a symbol denotes a documented change in the HIV envelope directed immune response. A shaded minus sign denotes a primary humoral response, and a shaded plus sign a secondary humoral response; a minus sign without shaded box denotes an antibody negative to specific epitope before and after immunization and a plus without shade an antibody positive to specific epitope before and after immunization, but no quantitative change. A dot with a shaded box indicates a new T-cell proliferative response to gp160 after immunization, and a dot without a circle the lack of a cellular response to gp160 (LSI < 3). LSI is the maximum lymphocyte stimulation index.)

In the Phase I trial, 6 of 15 volunteers who received the three-injection schedule were vaccine responders, as opposed to 13 of the 15 who received six injections. This suggests that more numerous immunizations may increase the immunogenicity in the original vaccine nonresponders. In addition to vaccine schedule, several other parameters were predictors of initial vaccine nonresponsiveness: lower CD4 values, higher levels of circulating IL-2R, higher values of spontaneous B-cell IgG production, and percent peripheral blood mononuclear cells (PBMC) cultures positive for HIV by Day 7. As a group, volunteers who demonstrated greater immunological damage (as determined by CD4 count and degree of B-cell and T-cell activation) and higher viral burden (as determined semiquantitative viral cultures), were more likely to be vaccine nonresponders.

All 10 vaccine nonresponders have completed the 6-injection schedule. Nine of the ten demonstrated alterations in both their

humoral and cellular anti-HIV envelope immune responses for an overall trial response of 9%. These surprising results suggest that a majority of early stage patients are capable of broadening their immune defense directed against HIV if given adequate product. To date, every volunteer who has received a six-injection regimen has immunologically responded to the vaccine. Table 2 summarizes anti-HIV envelope immune responses observed in the continuation trial. Following vaccine therapy, greater than 90% of volunteers demonstrated antibodies directed against C1 (96%), V3 (93%), and 448C (92%). Figure 3 compares vaccine-induced anti-envelope responses in gp160-treated volunteers to those in untreated early stage infected control volunteers.

Duration of immune response was evaluated through the continuation of study of the original responders. Individuals who responded in the original Phase I trial were boosted at four-month intervals. With this schedule we have been able to

TABLE 2. ANTI-ENVELOPE IMMUNE RESPONSES POST rgp160 VACCINE THERAPY CONTINUATION TRIAL.

Epitope	AA Sequence	% Volunteers immune response present	
		Prevaccination	Postvaccination
Humoral response to rgp 160			
C1	49-123	12	96
C2	254-274	0	23
V3	300-340	46	92
C3	342-405	0	35
448C	453-518	69	92
C4I	579-605	100	100
CKEN	735-752	12	23
Cellular response to rgp160			
LSI rgp160 \geq 5		10	100
LSI rgp160 \geq 10		0	92

This summarizes anti-envelope immune responses in the 27 volunteers who have completed a minimum of six injections with rgp160. Prevaccination values were obtained at Day 0 prior to vaccination. Postvaccination values were obtained 30 days post six-injection regimen. (LSI is the maximum lymphocyte stimulation index.)

continue to boost both the humoral and cellular immune responses. Of particular interest, we have not seen any evidence to suggest the induction of tolerance. Figure 4 illustrates a typical antibody and cellular response associated with recurrent booster vaccination. Epitope 241 is a particularly clear example as individuals do not develop antibody during natural infection, thus the initial response is a primary conversion and continued response is more easily attributable to booster vaccinations. Typically, we see an initial response, decay of antibody, increase of antibody temporally with vaccination and subsequent antibody decay. Continued boosting appears to result in persistence of this low titer antibody. The proliferative response also seems to be maintained throughout the patient population with sequential boosters. The duration and magnitude of proliferative response varies among patients such that the four-month schedule may not be optimal for all volunteers. We continue to administer booster injections at intervals of 4 month, while we further explore how often individuals must be boosted to maintain the immune response.

The continuation trial continues to support early observations related to safety. No evidence of systemic or immune-specific toxicity has been noted. Although the study was not designed to assess efficacy, CD4 counts were carefully monitored throughout the trial. It is extremely intriguing that at the time of analysis original vaccine responders experience 2.8% decline, and all trial volunteers 8.5% in contrast to historical natural history experience of a 26.1% decline. These data demonstrate long-term (2-3 year) safety and hint at clinical benefit. An ongoing trial designed to evaluate clinical efficacy will provide further information regarding this important issue.

WHY DOES POST INFECTION VACCINATION BROADEN HIV-SPECIFIC ENVELOPE DIRECTED IMMUNE RESPONSES

Prior scientific consensus suggested that postinfection modification of specific antiviral immune responses by vaccination

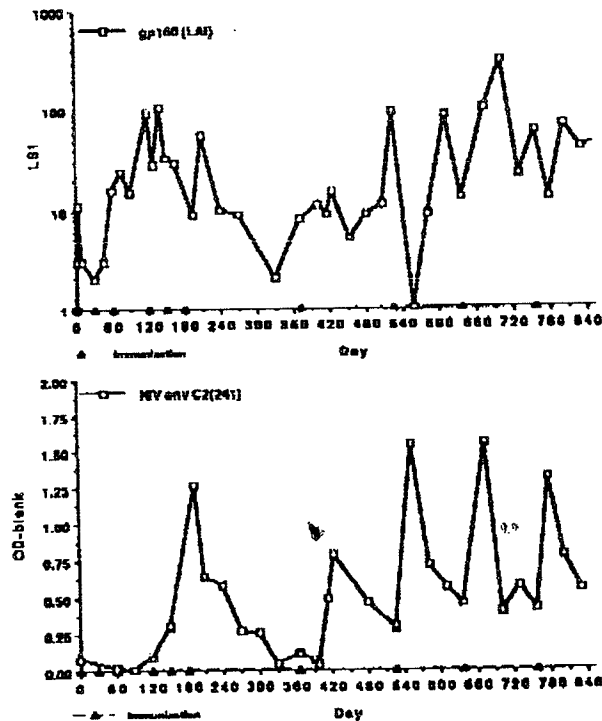


FIG. 4. A prototypic rgp160 (MicroGeneSys) vaccine responder, cellular, and humoral responses. (Panel A) Recombinant gp160 lymphocyte stimulation index relative to immunizations. Fresh PBMC are cultured in triplicate with serial concentrations of rgp160 (MicroGeneSys) throughout the vaccine trial. A temporal relationship between immunizations and enhanced proliferative responses is evidenced. (Panel B) Antihay reactivity in HIV envelop constant region 2 by peptide ELISA. Serial time point serum (1:100) is reacted with 241 (amino acid 241-272, LA1) peptide-coated plates. The ELISA optical density (405 nm) minus blank is plotted opposite trial days. (Pooled human serum reactivity \approx 0.05 O.D.) Generation of the new antibody response correlates with the primary immunization series. Subsequent boosting leads to persistence of the antibody (IgG₁).

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.

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

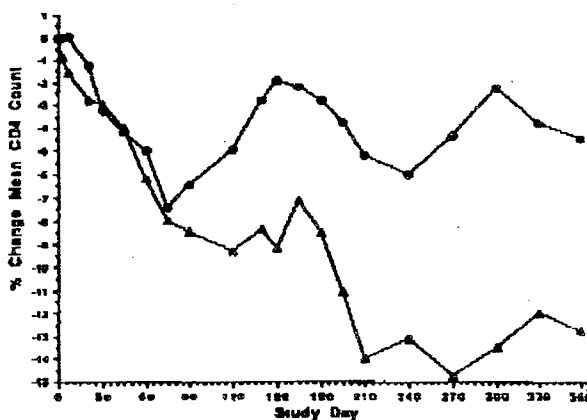


FIG. 5. Comparison of rgp160 humoral responders versus humoral nonresponders post vaccine therapy. The figure depicts the percent change from baseline mean CD4 count of volunteers who received rgp160 vaccine therapy. Circles represent mean CD4 change over one year in volunteers who developed novel anti-envelope 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.

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 Pepsican 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 induced 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.

Additionally, we have expanded our efforts to evaluate the feasibility of vaccine therapy in various patient populations such as patients with more advanced disease; patients treated with didanosine chemotherapy agents; newborns and children; HIV-infected pregnant volunteers; and developing world populations. We are also evaluating alternative products. Product variables may play an important role in determining the capability of a particular product to broaden the host-directed immune response. These include factors such as vaccine viral genotype, vaccine production expression system, the posttranslational modification of vaccine protein, protein conformation, vaccine formulation, and adjuvants. Each of these variables has the potential to have a significant impact on vaccine immunogenicity, safety and efficacy. Caution is required in extrapolating results attained utilizing one candidate gp160 or gp120 product to another. To further define the limits of postinfection vaccine therapy to modify and redirect the host immune response we continue to expand our Phase I evaluation of products to address the role of genomic diversity, protein glycosylation, and product formulation.

At present, the *in vivo* immunoregulatory consequences of anti-HIV responses remains unknown. Postinfection vaccination can enable us to define immunoregulatory mechanisms by expanding specific immune responses and measuring the consequence in terms of *in vivo* HIV replication or clinical disease progression. Such analysis would provide a strategy for the definition of protective immune response and may serve as a guide for development of a preventive vaccine. Additionally, if postinfection vaccination proves to be efficacious, it can conceivably provide an important and effective treatment option to modify the natural history of HIV infection and disease. HIV infection may not *a priori* be a chronic progressive pathogen in man.

As an experimental treatment strategy for HIV infection, vaccine therapy has several advantages over chemotherapy. First, carefully designed and executed trials not only provide information regarding potential treatment, it can increase our understanding of postinfection immunoregulatory mechanisms. As a treatment, the contrast between vaccine therapy and chemotherapy likely will be quite dramatic in terms of cost, ease of technology transfer to developing worlds, and the requirement for medical sophistication to administer, monitor, and treat iatrogenic complications. Second, vaccine therapy application in the setting of chronic infection will provide an alternative strategy for the development of candidate vaccines for prevention of HIV infection. Postinfection product performance provides criteria for product selection for evaluation of prophylactic efficacy and potentially will aid in the definition of natural anti HIV protective immune response. Additionally, should HIV-specific vaccine therapy prove clinically efficacious in augmenting postinfection control thereby reducing *in vivo* HIV replication, expression, and viral burden, such vaccination could provide an interim prevention strategy. Reduction of viral burden in the infected pool in a population could potentially reduce the kinetics of the epidemic in the noninfected population.

We encourage other investigators to join us in the investigation of postinfection vaccine therapy. Through our discovery of the potential of HIV-specific vaccine therapy we can illustrate and redefine fundamental immunological principles, delineate

the mechanisms of chronic viral expression and regulation, and ultimately harness this power and allow physicians to augment man's immune response to a chronic pathogen. If we are successful, the consequence of viral host interaction will no longer be dictated by nature but can be assisted by the power of science and wisdom of man.

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ALTERATION OF HIV SPECIFIC DNA AND RNA

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

* p=0.003 Fisher Exact (2-sided)

^a p=0.02 Fisher Exact (2-sided)

**Wilcoxon (Mann-Whitney) p < 0.05 (two-tailed)

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

Time	DNA*			RNA**		
	Arith Mean	Geom Mean	Range	Arith Mean	Geom Mean	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)

PROGRAM and ABSTRACTS



THE 32nd INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS and CHEMOTHERAPY

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860 Phase I Study of an HIV-1 gp120 Vaccine Combined with the Novel Adjuvant Emulsion, MF59/MTP-PE in Sero-negative Adults. J. KAHN*, D. CHERNOFF, F. SINANGIL, N. MURCAR, D. WYNNE, R. COLEMAN, N. HAIGWOOD, K. STEIMER and C. DEKKER. University of California San Francisco, and Chiron Corp., Emeryville CA.

A phase I randomized double-blind study was performed to determine safety and immunogenicity in HIV-seronegative adults of three injections of a vaccine composed of 25 µg of recombinant HIV gp 120 antigen combined with MF 59 emulsion containing a muramyl tripeptide covalently linked with dipalmitoyl phosphatidylethanolamine (MTP-PE) in a dose escalation format. The vaccine antigen is recombinant gp 120 from the SF2 strain of HIV-1, expressed in Chinese hamster ovary cells. The gp 120 vaccine is fully glycosylated and exhibits CD4 binding activity. Forty-two healthy HIV-seronegative adult men and women, with normal laboratory studies and without identifiable high-risk behavior for HIV infection were vaccinated. Vaccination occurred at day 0, 1 month and at 6 months. Each vaccine contained MF59 emulsion and each subject received MTP-PE (µg) dosing as follows: Group 1 (0) MTP-PE; Group 2 (1) MTP-PE; Group 3 (10) MTP-PE; Group 4 (50) MTP-PE; Group 5 (10) on the first immunization and (0) at month 1 and 6; Group 6 (100) MTP-PE at initial immunization and 0 on month 1 and 6. Two subjects in each group were randomized to receive placebo antigen while 6 received gp 120.

All subjects have received at least the initial vaccination. To date, subjects tolerated vaccination well. Symptoms reported include mild muscle aches, headache, low grade fevers. Antibodies appear to develop in a dose dependent manner with increasing antibody ELISA titers to gp 120 with increasing dose of MTP-PE (after 2 immunizations titer range: 400-12,000). Six subjects (25%) in groups 1, 2, 3 and 4 have developed HIV neutralizing antibodies after 2 immunizations (Titer: 30-270).

Initial information suggests that this candidate HIV vaccine is well tolerated and immunogenic. The safety and immunogenicity will be presented. The role of MF59 and MTP-PE in will also be discussed.

861 Do Geographically Divergent HIV-1 Isolates Generate Cross-reactive Neutralizing Antibodies? J. MASCOLA*, S. GARTNER, C. GROENENDAAL, F. MCCUTCHAN, A. FOWLER, K. WAGNER AND D. BURKE. The Military Medical Consortium for Applied Retroviral Research, Rockville, MD.

HIV-1 exhibits considerable genetic diversity, especially among isolates from different geographic regions. The immunologic significance of this diversity has not been well characterized. We have begun to perform cross neutralization (NT) studies using virus and plasma obtained from several HIV infected Thai (T) and United States (US) patients. Viral isolation and (NT) was performed using normal human T-lymphoblasts. PCR fingerprint and sequence analysis demonstrated the Thai and US isolates to be genetically distinct. NT assays performed with 100 TCID50 of virus are reported below. Results are expressed as the reciprocal titer giving a 50% reduction in p24 antigen. ND indicates not done.

(Viral Isolates)				
Plasma	US1	US2	US3	T244
US 1	ND	40	160	50
US 2	40	ND	98	36
US 3	160	80	ND	80
US 4	80	160	210	86
T 241	<10	<10	14	80
T 239	10	<10	<10	114
T 245	<10	10	14	114
T 235	10	<10	10	10

In these studies, US plasma neutralize US viral isolates and show some cross-reactivity with the Thai isolate. In contrast, Thai plasma neutralize the Thai virus but do not neutralize US isolates. These studies may have significance for development of HIV vaccines.

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HIV Vaccine Therapy: Phase I Safety and Immunogenicity using gp160: Three Year Follow-up REDFIELD RR*, BIRX DL, VAHEY M, JOHNSON S, LOOMIS L, MICHAEL N, POLONIS V, VANCOTT T, DAVIS C, BRUNDAGE J, SMITH G, BURKE DS and the Military Medical Consortium for Applied Retroviral Research WRAIR, HMFJ, MicroGeneSys

Twenty-eight of 30 original phase I volunteers agreed to re-enroll in a continuation trial. (NEJM Jun 91, Redfield). Initial vaccine responders received 160ug booster injection every 4 months; and vaccine nonresponders received 640ug on days 0, 7, 30, 60, 90 and 120, then every 4 months. Follow up has continued for 24 to 36 months post initial vaccination. All non-responders have completed rollover injection series; 9 of 10 have demonstrated both new humoral and cellular HIV envelop responses. All responders continue to demonstrate increases in humoral and cellular responses with booster injections. Further characterization of anti-envelope response demonstrates the capability of this III_b-based envelope product to induce broad cross reactivity anti-envelope antibody against multiple isolates including Northern American, Chiang Mai homologous wild type isolates (MN, III_b, Chiang Mai). Additionally, broad cross reactive T cell recognition was induced as assessed by T cell proliferation to multiple envelope sources (III_b, MN, SF2; Chiang Mai pending). We have applied a recently developed quantitative cellular-based RNA PCR technique (J Virology, Jan 92, Michael). Preliminary data demonstrates an antiviral effect as assessed by a reduction in genomic HIV RNA expression per infected PBMC. Toxicity remains limited to local reactions post immunization. We concluded that rgp160 vaccine is safe and immunogenic in patients with early HIV infection. A majority (97%) of early stages volunteers developed and maintained novel, broad (viral strain cross reactive), humoral and cellular responses not induced as a consequence of natural infection. These results implicate that antigen variation does not into limitations of immunogen recognition. In addition, the reduction of in vivo HIV expression supports an antiviral effect of this therapeutic strategy. A Phase II double blind placebo controlled trial was begun in November of 1990 to assess clinical efficacy.

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gp160 Vaccine Therapy in An HIV-1 Infected Patient is Associated with a Fall in Viral Burden, Antigenic Shift, and Viral Regulatory Sequence Mutations. MICHAEL*, N.L., VAHEY, M., CHANG, G., COOLEY, J., EHRENBERG, P., RUDERMAN, J., BIRX, D., and REDFIELD, R. Walter Reed Army Institute of Research and H. M. Jackson Foundation, Rockville, MD

We have recently demonstrated the feasibility of vaccine therapy with recombinant gp160 to broaden the host immune response to HIV-1 envelope. We now report data on the first patient for which parallel studies of viral load and DNA sequencing have been performed. This patient has shown a progressive fall in HIV-1 viral load of over one log unit during the study period by a quantitative PCR assay of peripheral blood mononuclear cells. Diminution in viral RNA preceded that for proviral DNA. This reduction in viral load has persisted for over two years without evidence for clinical progression or fall in CD4+ T-cell count. Direct DNA sequence analysis of the envelope and long terminal repeat regions from this patient was performed. Elevated viral burden was associated with a typical V3 loop tip sequence GPGRAFV and conserved transcriptional control regions. Alleles from a time point with lower viral burden showed a shift to a highly unusual GPGGSAFY sequence and the accumulation of extensive mutations in transcriptional control regions (especially SP1 sites I, II, and the TAR element).

These data support that the induction of novel immune pressure by vaccine therapy with gp160 has suppressed HIV-1 gene expression for an extended period in this patient resulting in the elimination of transcriptionally competent proviruses. We are determining if this mechanism applies to other patients in our trial who have also shown a fall in viral burden and lack of clinical progression.

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Cytokine Production by Vaccine-induced HIV-specific Cytolytic T Lymphocytes (CTL): Potential Effects on HIV-1 Replication and Clearance of Infected Cells.

R.C. BOLLINGER*, T.C. QUINN, A.Y. LIU, P.E. STANHOPE, W. PAVLAT, R. VIVEEN, M.L. CLEMENTS, R.F. SILICIANO. The Johns Hopkins School of Medicine, Baltimore and the NIAID, Bethesda, MD.

Certain cytokines, including tumor necrosis factor- α (TNF- α), have been shown to upregulate HIV-1 gene expression in cell lines infected with HIV-1. We investigated whether HIV-1-specific CTL induced by candidate AIDS vaccines release cytokines that affect HIV-1 gene expression in chronically infected cell lines. Cytokines produced by vaccine-induced CTL increased HIV-1 gene expression in chronically infected promonocytic and T lymphocytic cell lines. This was observed with both CD4⁺ and CD8⁺ CTL clones obtained from 3 volunteers immunized with candidate HIV-1 vaccines. HIV-1 upregulation was shown to be due to antigen-specific release of TNF- α . This TNF- α production by activated CTL could be inhibited by incubation with pentoxifylline. In addition to secreted TNF- α , transmembrane TNF- α was expressed by activated vaccine-induced CTL. Using a serine protease inhibitor which blocks the release of the secreted form of TNF- α , we showed that direct contact between activated CTL expressing transmembrane TNF- α and chronically infected cells could induce upregulation of HIV-1 gene expression.

Because TNF- α -induced upregulation of HIV-1 occurs in the vicinity of activated HIV-1-specific CTL, this data supports the suggestion that CTL-produced transmembrane and secreted TNF- α may facilitate local clearance of infected cells expressing levels of HIV-1 antigen that would otherwise be too low for CTL recognition.

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Influenza Immunization in HIV-infected Patients F. J. SORVILLO*, B. L. NAHLEN; K. M. FARIZO. Los Angeles County (LAC) AIDS Epidemiology Program and Centers for Disease Control, Atlanta GA

To determine the level and factors associated with influenza vaccine utilization among HIV-infected patients, a cohort of 1769 patients was assessed during the 1991-1992 influenza season. Influenza vaccine status, clinical and demographic data were obtained from medical records in 3 different outpatient clinics: a health maintenance organization (HMO), a public clinic and a private medical group. Fifty percent of the patients were white, 25% Latino and 18% black; 93% were male; 40% had an AIDS-defining condition; 81% reported male-to-male sex, 6% injecting drug use and 5% heterosexual contact. Patients receiving medical care from the HMO were more likely to receive influenza immunization (83%) than patients seen at the public clinic (42.3%) or the private facility (23.4%) (RR=2.0 and 3.6 respectively, p<.001). After controlling for clinic type, no associations were noted between influenza immunization and sex, race, age or risk category. A trend toward increasing immunization levels was observed with decreasing CD4 count (p<.01) however this association was a function of increased number of outpatient visits by patients with lower CD4 counts. No significant difference in the rate of bronchitis or pneumonia by vaccination status was observed, but small numbers precluded a valid assessment. In LAC, patients seen at the HMO had higher levels of influenza immunization. Although vaccine efficacy could not be assessed in this study, health care providers should increase efforts to provide influenza vaccine to HIV-infected patients.