

Perspective

Making Sense of the HIV Immune Response

A considerable amount of new information on HIV immunology has been generated over the past several years using novel techniques designed to assess HIV-specific immune responses. An understanding of the basic mechanisms involved in immune response and of these novel assessment techniques can aid the clinician in interpreting the literature in the area and considering the potential impact of new findings on clinical practice. Paul A. Goepfert, MD, provided an overview of HIV immunology at the Clinical Pathway of the Ryan White CARE Act 2002 All Grantee Conference held in Washington, DC, in August 2002.

Important advances in techniques designed to examine the immune response have allowed investigators to study HIV-specific immune responses in ways that were impossible only a few years ago. As a result, there has been a tremendous increase in the understanding of HIV immunopathogenesis, and clinically relevant findings from this research are becoming much more common. A basic understanding of applied immunology facilitates interpretation of new findings and their implications for clinical practice.

There are 3 basic levels of human defense against infections: surface barriers, innate defenses, and adaptive (or acquired) responses. This discussion focuses on adaptive responses, since most of the recent notable studies concerning HIV immunopathogenesis have provided information on this aspect of response.

When a viral pathogen such as HIV infects a cell, the host responds with both B-cell (humoral) and T-cell (cell-mediated) immune mechanisms. A sub-

set of the T cells, known as CD4+ or helper T cells, can be viewed as the orchestrators of the adaptive immune responses. The CD4+ T cells recognize foreign antigens bound to host proteins

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and aid B cells (eg, through production of various cytokines) in the production of antibodies. These antibodies have the potential to neutralize cell-free virus, thereby preventing subsequent viral infection of target cells in the body. They also participate in the lysis of infected cells by recruiting natural killer (NK) cells in a process termed antibody-dependent cell-mediated cytotoxicity. CD4+ T cells also aid in the stimulation and recruitment of another subset of T cells, the CD8+ T cells (eg, through production of cytokines). These CD8+ T cells, commonly referred to as cytotoxic T lymphocytes or CTLs, are able to target and lyse virally infected cells by recognizing foreign antigens bound by host proteins. Given that CD4+ T cells play a central role in the host response to pathogens and that infection of these cells by HIV leads to their destruction, it is easy to envision how HIV might destroy the immune system over time. Nevertheless, the exact details of this process are being elucidated in ongoing studies throughout the world.

Antibody Response

Anti-HIV antibodies have been of particular interest, given the potential for this arm of the immune system to be exploited in the development of an effective HIV vaccine. As noted, antibodies are able to bind cell-free virus and potentially prevent established infection in the challenged host. Such prevention seems ideal in combating HIV, which establishes a lifelong presence after initial infection. The hypothesis that inducing antibody response to HIV might permit effective vaccination is supported by 2 findings: (1) that the ability to induce antibody response is correlated with the efficacy of all commercially licensed vaccines (eg, those for measles, mumps, and rubella); and (2) that anti-HIV antibodies are protective against HIV infection in passive transfer experiments in nonhuman primates (see below). Optimism for attempts to develop anti-HIV vaccines that induce antibody response, however, is tempered by the fact that most effective vaccines prevent disease rather than the establishment of infection. Another caveat is that the correlation of antibody response with vaccine efficacy does not demonstrate a causal relationship between antibody response and prevention of infection or disease.

Although the presence of HIV-specific antibodies can be routinely detected by their ability to bind to proteins, it is generally believed that those antibodies that neutralize HIV—that is, neutralizing antibodies—are needed for an effective vaccine. Thus, studies in this area have focused on measuring neutralizing antibody responses. The basic neutralizing antibody assay is performed by mixing an HIV isolate with antibodies (usually in the form of serum; Figure 1). The excess, unbound antibodies are then washed off and the viral isolate is incubated with peripheral blood mononuclear cells (PBMCs). After a period of time in culture (about 5 days), the amount of

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virus growth is calculated by measuring the amount of HIV p24 antigen. By comparing the amounts of viral growth with and without antibodies, the percent neutralization can be calculated.

In animal experiments, a particular strain of virus or components of the virus that induce neutralizing antibody responses can be used as a vaccine to stimulate antibody response in vivo. Numerous passive transfer experiments have shown that when the serum of an immunized animal (which includes the elicited antibodies) is transferred to another animal, the latter animal is protected against a viral challenge when that challenge consists of a viral isolate that is identical to that used in the vaccine. Unfortunately, these studies have shown that when the same animal is challenged with a strain of virus that is different from the vaccine strain, no protection against infection is conferred. Most anti-HIV vaccines evaluated to date in both human and nonhuman investigations have thus only been able to induce antibody that neutralizes viruses very similar to the vaccine strain.

The significance of this problem is evident when the tremendous genetic diversity of HIV is considered—both among the range of virus types known to infect humans throughout the world and as reflected in genetic variants within a particular infected individual. For example, among the clade-B HIV-1 subtype prevalent in infections in the United States, there is a 30% genetic divergence among viral variants that can be reflected in the viral components targeted by the antibodies. In other parts of the world, a number of other clades of virus exhibit even greater genetic heterogeneity. Also, antibodies produced in response to vaccination fail to neutralize primary HIV isolates—that is, virus isolated from an infected individual. Despite these obstacles, various groups are attempting to design vaccines with the ability to neutralize a broad range of primary HIV isolates.

Cell-Mediated Immunity

Unlike antibody responses, which are generated in response to a foreign antigen alone, cell-mediated immune responses require that the T cells be presented with foreign antigen bound to

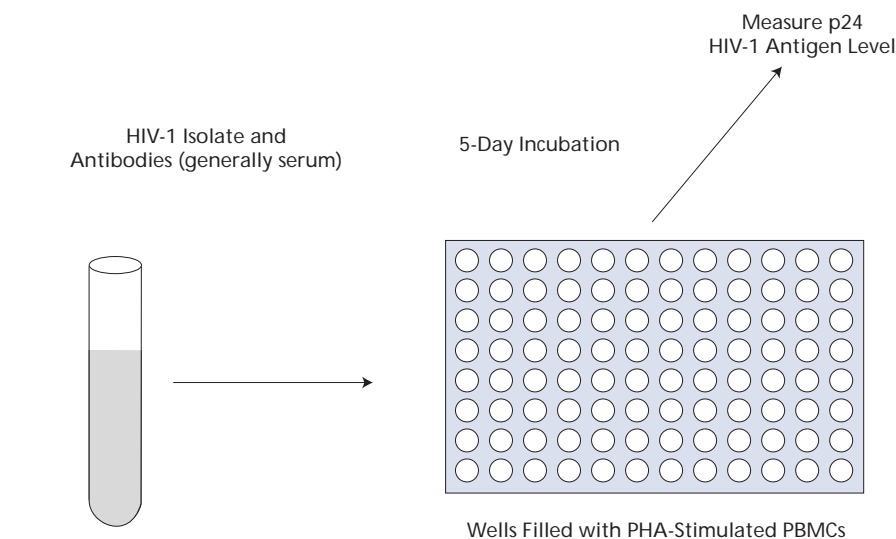


Figure 1. General schema for neutralizing antibody assay. The amount of virus growth is calculated by the amount of p24 antigen, and the percent neutralization, in turn, is calculated by comparing the amounts of virus growth with and without antibodies. PBMCs indicates peripheral blood mononuclear cells; PHA indicates phytohemagglutinin, a reagent used to stimulate T cells nonspecifically.

host proteins. These host proteins are known as the major histocompatibility complexes (MHCs), or human leukocyte antigens (HLAs). CD8+ T cells recognize foreign peptides of about 9 to 11 amino acids in length bound to MHC class I molecules, and CD4+ T cells recognize peptides of 11 to 20 amino acids in length bound to MHC class II molecules. MHC class I proteins are present on most human cell types, and they generally bind only those peptides that are synthesized within the cell itself. MHC class II proteins are found only on antigen-presenting cells, such as macrophages, dendritic cells, or lymphocytes. These cells are able to endocytose and process exogenous proteins into smaller peptides that are subsequently bound to class II molecules for presentation to and recognition by a CD4+ T cell. Therefore, as a general rule, CD8+ T cells respond to foreign antigens synthesized within a cell (ie, requiring infection of that cell), while CD4+ T cells respond to antigens encountered outside of the cell. Since viral infections feature both endogenous and exogenous antigens, both CD8+ and CD4+ T cell responses are elicited.

In the past, the measurement of cell-mediated immune responses has relied on the capacity of cells to proliferate and function in vitro. CD4+ T cells have been

measured by stimulating PBMCs with a desired protein (such as HIV p24) and leaving them in culture for several days (Figure 2). The cells are then placed in the presence of tritiated thymidine, and cellular proliferation is measured by the amount of incorporated thymidine. CD8+ T cell function is measured by mixing PBMCs with cells that have been infected with HIV or recombinant viruses that express HIV proteins (such as recombinant vaccinia viruses). The target cells are then radiolabeled with chromium. The amount of chromium released from the labeled cells is calculated and serves as a measure of the amount of cell lysis that occurs via activity of the CD8+ T cells.

In recent years, a more direct and easier method has been developed to measure both CD4+ and CD8+ T-cell responses. This assay relies on the detection of antigen-specific cytokine production by T cells. Both CD4+ and CD8+ T cells secrete interferon- γ (IFN- γ) in response to antigen-specific stimulation. In addition, both of these types of T lymphocytes are stimulated with peptides of 15 to 20 amino acids in length. Peptides (15-20 amino acids in length overlapping by 10-11 amino acids) are therefore used to stimulate PBMCs. These peptides are generally pooled to represent the various HIV proteins (Gag,

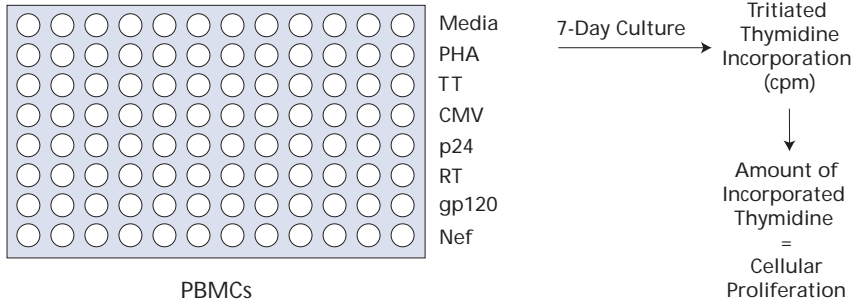


Figure 2. General schema for lymphoproliferative assay to measure CD4+ T cells. CMV indicates cytomegalovirus; PHA, phytohemagglutinin; PBMCs, peripheral blood mononuclear cells; RT, reverse transcriptase; TT, tetanus toxoid.

Pol, Env, etc), and positive responses can subsequently be determined down to the specific 15-to-20 amino acid peptide.

The detection of cytokine production is performed using a variety of methods: currently, the most common are the enzyme-linked immunospot (ELISPOT) assay and the intracellular cytokine staining (ICS) assay. The ELISPOT assay (Figure 3) detects the amount of IFN- γ secretion per cell represented as spots on a plate. These spots are then counted manually or with an automated counter. This assay generally does not discriminate as to whether the responses are due to CD4+ or CD8+ T cells and relies on a separate test to deplete either or both of these T-cell types to determine the cellular phenotype of the response. The ICS assay uses anti-IFN- γ antibodies conjugated to a fluorescence marker to detect intracellular production of this cytokine, and the staining of the IFN- γ is analyzed using a flow cytometer. Since the CD4+ and CD8+ T cells can be separately stained using individual antibodies conjugated with different fluorescence markers, the cell type responsible for the secretion of IFN- γ can be determined in the same test.

Another way to detect the presence of antigen-specific CD8+ T cells is by using a reagent known as a tetramer. The tetramer itself is synthesized by taking an MHC class I heavy chain and folding it with beta-2 microglobulin (β_2m) in addition to the foreign peptide of interest. This complex is then bound to a streptavidin molecule through a biotinylation site engineered into the MHC class I/ β_2m /peptide complex. The tetramer reagent derives its name from the fact

that it consists of 4 of these molecular complexes bound to the tetravalent streptavidin molecule, which is subsequently conjugated to a fluorescence marker. This tetramer can then be used to bind and identify a specific type of CD8+ T cell.

The tetramer will bind only CD8+ T cells that are restricted by both the MHC class I and the foreign peptide that was engineered into the tetramer. Therefore, although the tetramer reagent is extremely specific and will detect only 1 type of HIV-specific CD8+ T cell, it can be used to readily and easily detect the presence of these specific CD8+ T cells. The tetramer assay cannot detect the function of a cell. However, when it is combined with assays such as the ELISPOT or the ICS assay, which detect the production of cytokines, it is possi-

ble to detect both the presence and function of specific CD8+ T cells (Figure 4). Similar tetramer reagents are also being produced that have the capacity to detect specific CD4+ T cell responses, but these assays are not nearly as far along in development as the tetramer assays to detect CD8+ T cell responses.

Recent Findings in HIV Immunology

A number of recent findings have contributed to our understanding of HIV immunology and posed additional questions. For example, Schmitz and colleagues (*Science*, 1999) were interested in determining which HIV-related immune responses controlled viral replication. The investigators used an anti-CD8+ antibody to deplete the CD8+ T cells of rhesus macaque monkeys chronically infected with simian immunodeficiency virus (SIV). Prior to the depletion of CD8+ T cells, SIV replication was well-controlled in all of the monkeys. However, after the depletion of the CD8+ T cells, control of viral replication was lost. In some of these monkeys, the CD8+ T cells actually regenerated and, when they did, control of viral replication was regained. This study and others like it clearly show that the SIV-specific CD8+ T cells play an important role in controlling viral replication. Since SIV infection is a good model for HIV pathogenesis, such findings suggest

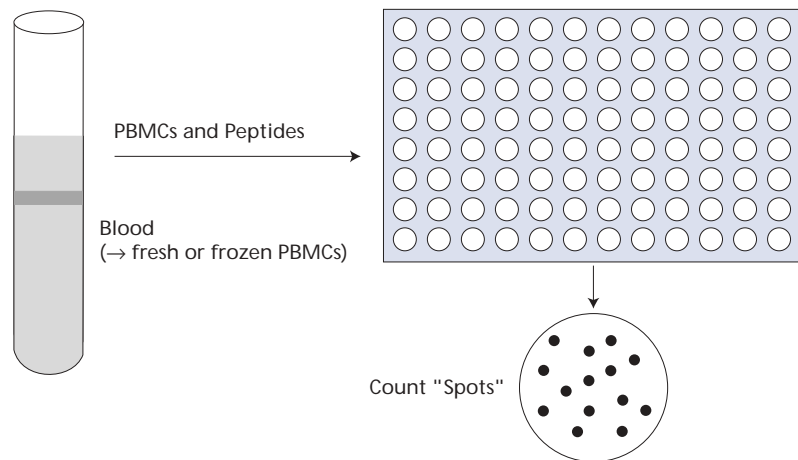


Figure 3. General schema for interferon- γ enzyme-linked immunospot (ELISPOT) assay. PBMCs indicates peripheral blood mononuclear cells.

that CD8+ T cells play a similar role in controlling viral replication in HIV infection. Additional studies demonstrating a temporal relationship between HIV-specific CD8+ T cells and control of viral replication in humans support this suggestion.

Given the important role that CD8+ T cells play in controlling viral replication, many investigators have attempted to develop vaccines that elicit HIV-specific CD8+ T-cell responses. Several groups have used SIV vaccines as a model for HIV vaccine development. Amara and colleagues (*Science*, 2001) observed a high level of SIV Gag-specific T-cell responses when monkeys were immunized with a DNA vaccine that encodes SIV-specific proteins followed by a recombinant modified vaccinia Ankara (rMVA) virus that encodes the same SIV-specific proteins. These monkeys were then challenged with SIV at 27 weeks after their vaccine boost. Although all of the monkeys became infected, the monkeys that received the SIV-specific vaccines exhibited control of viral replication and preservation of CD4+ T-cell counts. Monkeys that did not receive vaccine had high levels of viral replication and a precipitous drop in their CD4+ T-cell counts after SIV challenge. These and similar findings have encouraged hope for the development of a vaccine that elicits CD8+ T-cell responses. Although these vaccines may not protect against infection, they may protect against disease progression by controlling viral replication. HIV-infected individuals with controlled viral replication may also be at lower risk of subsequently transmitting the virus.

Another important study that focused on the immune responses to HIV was performed by Rosenberg and colleagues (*Nature*, 2000). These investigators had observed that HIV-specific CD4+ T-cell responses as measured by a lymphoproliferative assay were of high magnitude in individuals who were HIV-infected but did not exhibit progressive disease over long-term follow-up (long-term nonprogressors, LTNPs). These lymphoproliferative responses were not generally detectable in patients with chronic progressive infections. The investigators theorized that one reason virus was controlled in the LTNPs was that HIV-specific CD4+ T-cell responses

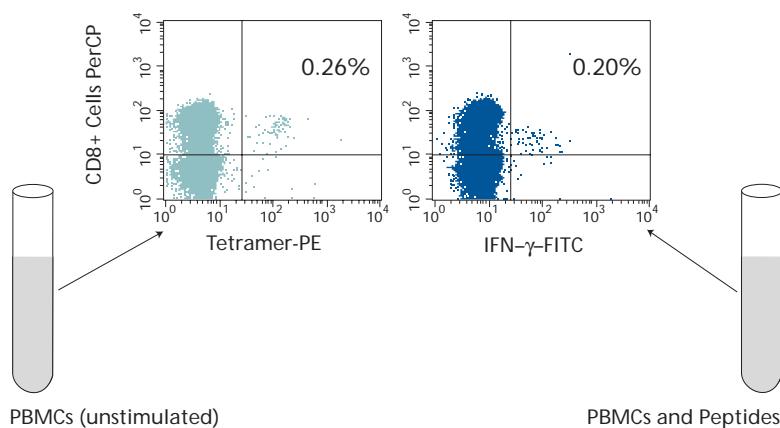


Figure 4. Tetramer assay showing that 0.26% of CD8+ T cells are specific for a peptide used in the assay (left) and stimulation of CD8+ T cells with the same peptide showing that most of these cells are functioning (right) as indicated by IFN-γ production. PerCP indicates peridinin chlorophyll markers; PE, phycoerythrin; FITC, fluorescein isothiocyanate (all types of fluorescence markers).

were preserved. They also believed that these CD4+ T-cell responses would be present in a person with acute viral infection but would diminish shortly thereafter.

The authors subsequently demonstrated that individuals identified with acute HIV infection who also began potent antiretroviral therapy had very high lymphoproliferative responses, the magnitude of which was comparable to that seen in LTNPs. When potent antiretroviral therapy was discontinued in these patients after at least 6 months of therapy, 3 of 8 patients exhibited control of viral replication (plasma HIV-1 RNA level <5000 copies/mL), and 4 of the 5 remaining patients exhibited control of viral replication after further rounds of starting and stopping antiretroviral therapy. It is important to note the limitations of this study, including the fact that it involved only a limited number of patients who were followed for short periods of time after treatment interruption. Much more work is needed in this area before firm conclusions can be made.

Another important limitation of the study by Rosenberg and colleagues is that the results have not been replicated in patients with chronic infection, as observed in numerous clinical trials. Additionally, there is no consistent evidence that immune responses are enhanced in chronically infected patients who undergo strategic treatment

interruptions (STIs). It is possible that STIs do not work in chronically infected individuals because these individuals have already lost their HIV-specific CD4+ T-cell responses. Rosenberg and colleagues hypothesized that HIV-specific CD4+ T cells may be preferentially infected and killed in early HIV infection. Therefore, although the absolute CD4+ T-cell counts may be relatively preserved after acute infection, the HIV-specific T cells are actually significantly depleted.

More recently, Douek and colleagues (*Nature*, 2002) reported findings consistent with the hypothesis proposed by the Rosenberg group. These investigators stimulated PBMCs with HIV-specific proteins or with proteins specific to cytomegalovirus (CMV), a well-controlled but chronic viral infection. They evaluated the secretion of IFN-γ and sorted the CD4+ T cells that secreted IFN-γ in response to the virus-specific antigens using a fluorescence-activated cell sorter. The sorted cells were then tested for the presence of HIV-specific DNA. These elegant studies showed that HIV-specific CD4+ T cells contain greater amounts of HIV-specific DNA and hence have a greater amount of HIV infection than do memory phenotype CD4+ T cells or CMV-specific CD4+ T cells. These differences were further enhanced when samples from patients who experienced a rapid viral rebound after undergoing an STI were evaluated (Figure 5). These studies therefore demonstrate that

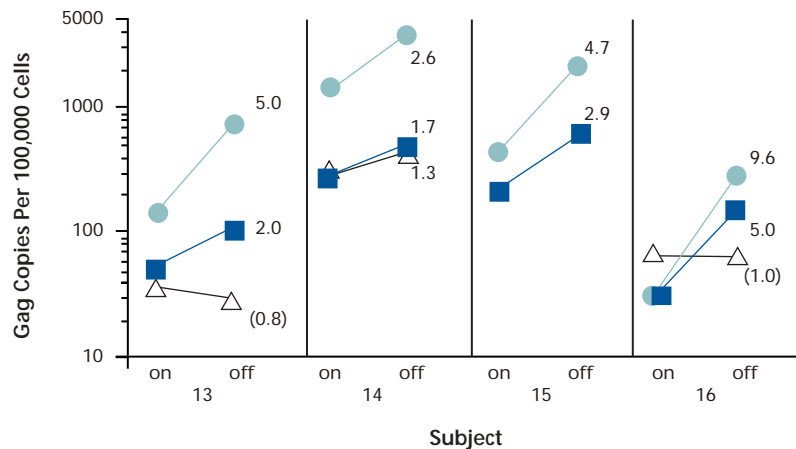


Figure 5. Viral DNA in HIV-specific CD4+ T cells (green circles), memory (CD45RO+) CD4+ T cells (blue squares), and cytomegalovirus-specific CD4+ T cells (white triangles) of infected subjects on and off antiretroviral therapy, showing preferential infection of HIV-specific CD4+ T cells after stopping potent antiretroviral therapy. The magnitude of change in viral DNA is shown. Adapted with permission from Douek et al, *Nature*, 2002.

although STIs can be beneficial in patients with acute infection if viral replication is controlled, HIV-specific CD4+ T cells may be sacrificed very quickly in individuals who do not maintain control of viral replication following STI. The latter situation may be particularly relevant in chronically infected patients.

Model of HIV Immunopathogenesis

In summary, a variety of data demonstrate that both CD4+ and CD8+ T cells are extremely important in controlling HIV infection. Preventive vaccines are now being designed to elicit HIV-specific CD8+ T cells in the hope of preventing disease progression. In a small number of individuals who began treatment shortly after acute HIV infection, HIV-specific CD4+ T-cell responses were preserved. In addition, these CD4+ T-cell responses seem to be important in controlling viral replication after subsequent discontinuation of antiretroviral therapy. However, it has also been found that when discontinuation of antiretroviral therapy leads to loss of virologic control, HIV-specific CD4+ T cells are preferentially infected and depleted when compared with the CD4+ T cells of other antigen specificities.

Based on this and other information, a model of HIV immunopathogenesis

based on virus-specific immunology can be hypothesized. In this model, an individual is infected with HIV, and CD8+ T cells with the specific help of CD4+ T cells are able to efficiently neutralize the virus infecting the CD4+ T cells. However, it is now quite clear that the virus rapidly mutates, with mutants that reduce recognition by CD8+ T cells being favored in the context of the selective pressure from CD8+ T cell activity. The mutated proteins or other HIV proteins can still subsequently serve as targets for CD8+ T cells. However, the CD8+ T cells are now acting without the presence of CD4+ T cells, because these HIV-specific CD4+ T cells have been lost as a direct consequence of infection. This presumably allows disease progression to occur; in the absence of antiretroviral therapy, CD4+ T cells are gradually depleted and CD8+ T-cell response is lost, with the infected individual ultimately developing clinical disease.

It must be noted that this model is not fixed, reflects the author's own hypothesis, and in fact is controversial in the field of HIV research. It undoubtedly will change and be reshaped with future experiments. In addition, all of the information gleaned from study in this area will enhance our understanding of HIV immunopathogenesis and lead to improved strategies in preventing and treating HIV disease.

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IAS–USA Recommendations for Management of Metabolic Complications in HIV-1 Infection. Schambelan M, Benson CA, Carr A, et al. *JAIDS*. 2002.

Antiretroviral therapy has substantially reduced mortality for HIV-1-infected patients. However, an increasing number of adverse metabolic effects associated with such treatment are emerging. The mechanisms underlying these complications and their relationship to specific antiretroviral drugs remain unclear, and definitive data on managing metabolic effects are scarce. Nevertheless, the potential long-term risks and effects on quality of life are concerning to HIV clinicians and their patients.

In light of these concerns, the International AIDS Society–USA appointed a 12-member panel representing international expertise in HIV-1 patient care, antiretroviral therapy, and endocrine and metabolic disorders. The panel reviewed relevant data and developed recommendations for the clinical management of metabolic complications associated with antiretroviral therapy and HIV disease itself. Recommendations were developed by group consensus with emphasis on results of prospective, randomized, controlled trials in

HIV-1-infected patients where possible, although such data are scant at this time. The panel also considered expert opinion and data from ongoing trials, epidemiologic studies, laboratory-based investigations, and studies of similar complications in individuals without HIV-1 infection.

The IAS–USA panel concludes that controlled studies to determine the incidence, cause, risk factors, and most appropriate treatments for metabolic complications in HIV-1 infection are urgently needed. The recommendations are published in the November 1, 2002, issue of the *Journal of Acquired Immune Deficiency Syndromes*. The full text of the article is available online at www.iasusa.org/pub/metcomp.html for a limited time or may be accessed on the *JAIDS* Web site (www.jaids.com) with a *JAIDS* subscription or pay-per-view fee.

The recommendations address diagnostic assessment, monitoring, and treatment for 5 areas.

Glucose Intolerance and Diabetes Mellitus

- **Assessment and Monitoring:** Assess fasting glucose before initiation of or switch to a protease inhibitor (PI)-containing antiretroviral regimen, 3 to 6 months after starting or switching this therapy, and annually thereafter. In patients with risk factors for type 2 diabetes or those with severe body fat changes, 75 g of oral glucose may identify impaired glucose tolerance.
- **Treatment:** Encourage weight loss for overweight patients. For persistent fasting hyperglycemia, follow diabetes guidelines established for the general population. If drug therapy is indicated, preference should be given to insulin-sensitizing agents such as metformin (except for patients with renal disease or history of lactic acidemia) or thiazolidinediones (except for patients with preexisting liver disease). If possible, consider avoiding PIs in initial regimens for patients with preexisting glucose intolerance or diabetes.

Lipid and Lipoprotein Metabolism Abnormalities

- **Assessment and Monitoring:** A fasting lipid panel (total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol, and triglyceride levels) should be obtained before initiating or changing antiretroviral therapy, 3 to 6 months after therapy initiation or switch, and annually thereafter.
- **Treatment:** Follow National Cholesterol Education Program (NCEP) III guidelines for assessing cardiovascular risk and dietary and lifestyle alterations to lower cholesterol and triglyceride levels. If possible, avoid PIs in patients with preexisting cardiovascular risk, high lipid levels, or family history of hyperlipidemia. Follow NCEP III guidelines as a framework for use of lipid-lowering agents. If drug therapy is indicated, fibrates are recommended as initial therapy for hypertriglyceridemia, and pravastatin or atorvastatin preferred for patients with elevated cholesterol. If combination therapy is indicated, begin with a statin and add a fibrate if there is insufficient response after 3 to 4 months.

Body Fat Distribution Abnormalities

- **Assessment and Monitoring:** No specific technique can currently be recommended for assessment and monitoring of body fat dis-

tribution changes, as no method has demonstrated sufficient sensitivity, specificity, or predictive value. Measurement of waist circumference, which can be done easily and inexpensively in a clinical setting, has been recommended in the NCEP III guidelines.

- **Treatment:** Based on the available evidence, no therapies for fat distribution abnormalities in the absence of other metabolic complications can be routinely recommended. The panel discussed existing data surrounding several potentially promising therapies that are now being tested in controlled clinical trials.

Lactic Acidemia

- **Assessment and Monitoring:** Routine measurement of lactic acid levels is not recommended. Lactic acid levels should be monitored in patients receiving nucleoside reverse transcriptase inhibitors (nRTIs) who have symptoms consistent with lactic acidemia or who are pregnant. If alternative nRTIs are resumed after interrupting antiretroviral therapy for lactic acidemia, lactate levels should be monitored every 4 weeks for at least 3 months.
- **Treatment:** Discontinue antiretroviral therapy for all patients with confirmed lactate levels above 90 mg/dL (10 mmol/L) or for symptomatic patients with confirmed lactate levels above 45 mg/dL (5 mmol/L). No intervention apart from nRTI cessation is recommended. Restart combination therapy with nonnucleoside reverse transcriptase inhibitors and PIs after lactate levels normalize and symptoms resolve.

Osteopenia, Osteoporosis, and Osteonecrosis

- **Assessment and Monitoring:** Routine screening for osteoporosis or osteonecrosis is not recommended. For patients with bone or joint pain, a radiographic examination of the involved bone and assessment of the contralateral joint are recommended.
- **Treatment:** Surgical resection of the bone is the only effective therapy for symptomatic osteonecrosis. If scanning demonstrates osteoporosis or if a pathologic fracture occurs in the setting of osteoporosis, consider bisphosphonate therapy.