

Advances in Asthma, Allergy and Immunology Series 2004: Basic and clinical immunology

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This review highlights some of the most significant advances in basic and clinical immunology that were published from August 2002 to December 2003, focusing on manuscripts that appeared in the Journal. Articles selected were those considered most relevant to Journal readers. With regard to basic immunology, this report includes articles describing FcεRI expression in mucosal Langerhans cells and type II dendritic cells, mechanisms of T_H1 and T_H2 regulation, the role of Foxp3 in the development of CD4⁺CD25⁺ regulatory T cells, and the increasing importance of Toll receptors in immunity. Articles related to clinical immunology that were selected include the first report of lymphocyte subsets values from a large cohort of normal children; the description of new genetic defects in primary immunodeficiencies; a description of the complications of gene therapy for X-linked severe combined immunodeficiency; a report of 79 patients with hyper-IgM syndrome; a report of the mechanism of action and complications of intravenous immunoglobulin; a report of new approaches for immunotherapy; and an article on advances in HIV infection and management, including a report of defensins, small molecules with anti-HIV properties. Also summarized is an article that studied the immune system during a prolonged stay in the Antarctic, a model for human studies on the effect of environmental conditions similar to space expeditions. (J Allergy Clin Immunol 2004;114:398-405.)

Key words: Immunology, regulatory T cells, immunodeficiency, HIV, gene therapy, lymphocyte subsets

The body of knowledge in basic and clinical immunology grows exponentially every year. This article summarizes some of the significant progress achieved from October 2002 to December 2003. Because of the space limitations of this article, it is not possible to report all of the significant progress in that period. The authors present

Abbreviations used

ADA:	Adenosine deaminase
CVID:	Combined variable immunodeficiency
DC:	Dendritic cell
hBD:	Human β-defensin
ICOS:	Inducible costimulator of activated T cells
IVIG:	Intravenous Ig
LC:	Langerhans cell
NK:	Natural killer
PLA2:	Phospholipase A2
SCID:	Severe combined immunodeficiency
SLAM:	Signaling lymphocyte activation molecule
TLR:	Toll-like receptor
Trif:	Toll/IL-1 receptor domain-containing adaptor inducing IFN-β
UNG:	Uracil N-glycosidase
XSCID:	X-linked severe combined immunodeficiency

the advances considered most relevant to the readers of the Journal.

BASIC IMMUNOLOGY (TABLE I)

Modulation of T_H1 and T_H2 cells

The roles of T_H1 and T_H2 cells in the pathogenesis of occupational asthma were studied in mice by using a protocol to develop lung inflammation after skin sensitization with di-isothiocyanates, the chemical that most commonly induces occupational asthma in human beings.¹ Eosinophils in bronchoalveolar lavage were significantly increased in the treated mice compared with control mice. T_H2 cytokines IL-5 and IL-13 were also elevated. However, the eosinophilia did not occur when this protocol was applied to mice of strains different from Balb/c, including CBA and C57BL6. All mice strains developed skin sensitivity, suggesting that genetic factors are involved in the induction of airway inflammation with these chemicals.

Cates et al² introduced purified ragweed extract intranasally to evaluate airway inflammatory responses by using a novel mouse model. They were able to show increased eosinophils in bronchoalveolar lavage and increased lymph node cells and splenocytes expressing IL-4, IL-5, and IL-13. The addition of GM-CSF greatly increased these effects and also induced the presence of monocytes in bronchoalveolar lavage. This is a useful

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airway inflammation model for studying cytokines and the role of antigen presenting cells induced by aeroallergens.

Knockout mice are a useful means to understand protein function. Kusunoki et al³ studied determinants of T_H1 and T_H2 regulation in Id2 knockout mice. Id2 is a transcription factor from the helix-loop-helix family. Id2 knockout mice lack lymph nodes and exhibit elevated T_H2 cytokines and IgE levels and decreased T_H1 cytokine expression.⁴ This study found that naive CD4 T cells from Id2 knockout mice were able to differentiate normally into T_H1 cells *in vitro*. CD8 α /CD11b dendritic cells, associated with induction of T_H1, were remarkably low. The authors suggest that Id2 protein is important for regulating T_H1/T_H2 balance by inducing the differentiation of CD8 α ⁺ dendritic cells.

IL-21, a recently described cytokine with unique effects in T_H1 and T_H2, was reviewed in the Journal by Habib et al.⁵ The receptor for this cytokine is composed of a type I cytokine receptor, IL-21R, and the common cytokine receptor γ -chain that is shared with IL-2, IL-4, IL-7, IL-9, and IL-15 receptors. IL-21 knockout mice display decreased IgG responses to antigen stimulation and increased IgE levels. IL-21 induces increased T-cell and natural killer (NK) cell activity. It has a role in enhancing antitumor activity and in regulating the activation of T_H2 responses. The clinical use of IL-21 to enhance T_H1 activity without overstimulating T_H2 responses is being evaluated.

CD4⁺CD25⁺ regulatory T cells

CD4⁺CD25⁺ regulatory T cells express the IL-2 receptor surface marker CD25 and constitute about 10% of the CD4⁺ T cells. Their role in preventing autoimmune conditions, transplant rejection, and inflammatory bowel disease has been established (Fig 1).⁶ In 2003, Hori et al⁷ demonstrated that the transcription factor Foxp3 was essential for the differentiation of these regulatory T cells. When Foxp3 was mutated, mice developed autoimmune diseases similar to those in mice lacking regulatory T cells. They also showed that naive T cells acquired regulatory T-cell functions if Foxp3 was overexpressed. These modified cells were able to inhibit the development of inflammatory bowel disease in a mouse model. Fontenot et al⁸ and Khattri et al⁹ independently reached similar conclusions. Mutations of Foxp3 in human beings result in a condition called IPEX (immune dysregulation, poly-endocrinopathy, enteropathy, X-linked syndrome), characterized by autoimmune endocrinopathy, type I diabetes, inflammatory bowel disease, and severe atopy.¹⁰

Umetsu et al¹¹ reviewed the role of different immune regulatory cells in allergic disease and asthma, including CD25⁺CD4⁺ T cells. Mouse models and data from human beings suggest that these regulatory cells and T_H3 cells (CD4⁺ T cells that produce TGF- β) are important in the control of asthma, reducing the inflammation induced by cytokines and immune cell activation. Bellinghausen et al¹² showed that CD4⁺CD25⁺ cells from 7 atopic patients and 6 nonatopic patients equally inhibited T_H1

TABLE I. Key advances in basic immunology

- (1) Genetic factors may influence the pathogenesis of occupational asthma
- (2) Id2 protein participates in the induction of T_H1 responses
- (3) Foxp3 is responsible for the development of CD4⁺CD25⁺ regulatory T cells
- (4) Fc ϵ RI is expressed in type II DCs and in oral mucosa LCs
- (5) Trif is a signal transduction protein that mediates IFN- β secretion in response to TLR3 and TLR4 stimulation

and T_H2 responses, and although these cells were equally present in atopic and nonatopic patients, a subset of the atopic patients had regulatory cells that predominantly suppressed only T_H1 responses.

Dendritic cells

Dendritic cells (DCs) were investigated in 2 of the studies published in the Journal. DCs found in the epidermis are called *Langerhans cells* (LCs) and are characterized by the presence of CD1a, Fc γ RII, MHC molecules class I and II in the cell surface, and Birbeck granules in the cytoplasm. Allam et al¹³ took skin and oral mucosa samples from normal adult volunteers undergoing surgery to compare LCs obtained from those tissues. They reported increased expression of MHC class I and II, Fc ϵ RI, and costimulatory molecules in LCs obtained from oral mucosa, with subsequent increased ability to stimulate T cells. This finding may represent increased activation in oral mucosa LCs, considering their increased antigen accessibility compared with skin epidermis. The authors also proposed the role of LCs in oral tolerance because of the increased Fc ϵ RI expression. Foster et al¹⁴ studied DCs obtained from peripheral blood and tonsil lymphoid tissue from normal volunteers undergoing elective surgery. They found that the Fc ϵ RI expression levels in DC type I and type II were similar, and correlated with IgE levels in serum. This was surprising, because it was previously reported that Fc ϵ RI was absent in DC type II. The expression of Fc ϵ RI in both DC type I and type II subsets was higher in patients with allergy and patients with asthma. The study findings are relevant to efforts in the modulation of Fc ϵ RI expression for therapeutic purposes, which could affect both sensitization and effector phases.

Toll-like receptors

In the last 5 years, we have learned the importance of Toll-like receptors (TLRs) in the control of infections.¹⁵ Human immune cells express 10 different TLRs that recognize specific molecules present in pathogens, such as LPSs. TLR binding activates signaling cascades and cytokine secretion and initiates the development of specific immune responses. One intracellular pathway, mediated by the adaptor protein MyD88, was described as common to all TLRs and as activating cytokine secretion. A second pathway mediated by the Mal/TIRAP protein is involved in signals from TLR2 and TLR4. However, some TLR functions have been found to be independent of

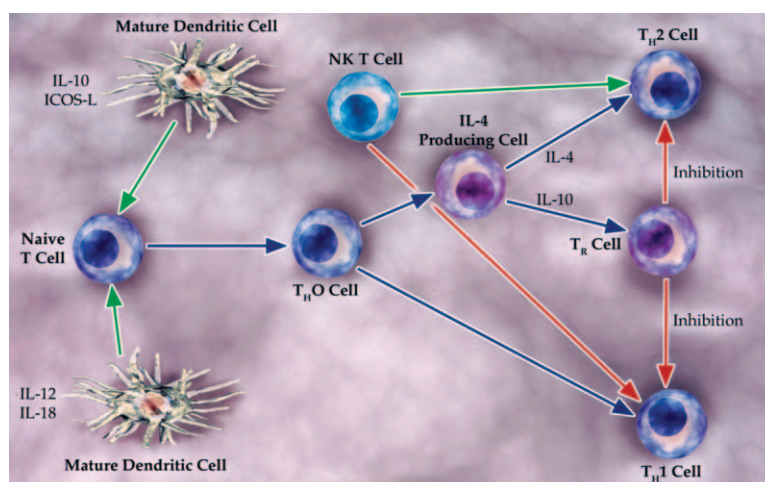


FIG 1. Regulatory T cells (T_R) and NK T cells regulate the differentiation (blue arrows) of T_H1 and T_H2 cells. DCs producing IL-12 and IL-18 enhance (green arrows) T_H1 cell development, whereas DCs expressing ICOS ligand and producing IL-10 enhance the development of T_H2 and T_R cells. T_R cells inhibit (red arrows) both T_H1 and T_H2 cells. NK T cells have critical effects on T_H1/T_H2 cell differentiation. Reprinted with permission from *The Journal of Allergy and Clinical Immunology*, Vol 112, Umetsu et al, "Regulatory T cells control the development of allergic disease and asthma," pp 480-7, Copyright 2003, with permission from The American Academy of Allergy, Asthma and Immunology.

TABLE II. Key advances in clinical immunology

- (1) Normal values for lymphocyte subsets from a large series have been reported
- (2) New genetic defects in primary immunodeficiencies have been described, including *ICOS*, *CD3 δ* , *UNG*, *CXCR4*, *STAT-1*, and *IRAK4*
- (3) IL-12R β 1-deficient patients have relatively good prognoses
- (4) Gene therapy for ADA-SCID has been proven successful
- (5) Airway hyperreactivity in asthma may not be mediated by T_H2 cytokines
- (6) Early SLAM expression may be an indicator of response to immunotherapy
- (7) IL-4 antisense oligonucleotide inhibits IL-4 synthesis and decreases T_H2 -mediated inflammation
- (8) A new long-term study suggests a very slow rate of HIV-1 decay in plasma, with a $t_{1/2}$ of 44.2 months
- (9) An anti-HIV cell entry inhibitor, Enfuvirtide, has proven beneficial in phase III trials when used in addition to optimized antiretroviral therapy

gens and immune cells, and the development of specific immune responses triggered by the innate immunity.

CLINICAL IMMUNOLOGY (TABLE II)

Normal values for lymphocyte subsets in children

Normal reference values for lymphocyte subsets in children in the United States previously had been available only from small series of subjects. The changing ontogeny of T cells in children requires having value sets for different age brackets. Shearer et al¹⁹ collected data from 807 normal children as a control reference for HIV-infected children. This population, which included minorities, was recruited from 29 pediatric US centers and divided into 7 strata: 0 to 3 months of age, 3 to 6 months, 6 to 12 months, 1 to 2 years, 2 to 6 years, 6 to 12 years, and 12 to 18 years. There were significant variations in the values of T-cell subsets according to age, but only a few differences regarding race or ethnicity. The use of this set of normal values can be extended to studies on a variety of immune-based diseases in children, such as leukemias and rheumatoid arthritis.

Primary immunodeficiencies

The report on normal lymphocyte values has proven useful in immunodeficiency studies, such as the article on T-cell stability in partial DiGeorge syndrome.²⁰ Patients with partial DiGeorge syndrome present with decreased but significant numbers of T cells and variable immunodeficiency. Forty-five of these patients were studied long-term to determine whether T-cell counts decreased over time and the patients developed more severe immunodeficiency. T-cell counts decreased with an average rate

MyD88 and Mal pathways.¹⁶ One of these functions is IFN- β secretion induced by activation of TLR3 and TLR4. Working with 2 different mouse models, Hoebe et al¹⁷ and Yamamoto et al¹⁸ found that there was a third pathway in TLR signal transduction that was mediated by Toll/IL-1 receptor domain-containing adaptor inducing IFN- β (Trif). Hoebe et al¹⁷ generated mice with high susceptibility to viruses by using chemical mutagenesis and screening for mutant strains. Then they showed that the mutation responsible for this susceptible phenotype was in the Trif protein. Yamamoto et al¹⁸ first generated a Trif-deficient mouse, and then showed that cells from these mice had severe impaired secretion of IFN- β and other inflammatory cytokines. These studies contribute to a better understanding of the interaction between patho-

of 0.7% per month during the first year and increased 0.1% per month from 12 to 120 months of age, suggesting that T-cell populations were relatively stable, and more severe T-cell deficiency was not anticipated in these patients. Further studies are needed to determine whether precautions for T-cell deficiency can be discontinued safely in patients with mild to moderately reduced T-cell numbers.

The presentation and evolution of patients with X-linked hyper-IgM syndrome in the United States were reported in a collaborative effort that included a large number of patients.²¹ Seventy-nine patients from 60 families were identified after collecting data contributed by members of 7 US academic medical societies. X-linked hyper-IgM syndrome is caused by mutations of the CD40 ligand gene. This gene is expressed in the T-cell surface to induce lymphocyte activation and Ig class-switch recombination in B cells. Patients have normal or elevated IgM levels and decreased levels of IgG, IgA, and IgE. They also have a variable defect in T-cell proliferation. These patients showed clinical presentation in the first year of life in 75% of cases. Pneumocystis pneumonia was the most common clinical diagnosis at presentation, and *Cryptosporidium* was an important cause of diarrhea and of sclerosing cholangitis. Neutropenia was present in 63% of patients.

In 2003, new genes causing defects of the immune system in human beings were described. Grimbacher et al²² screened 32 patients with combined variable immunodeficiency (CVID) for mutations in inducible costimulator of activated T cells (ICOS). CVID is the second most common primary immunodeficiency, and previous attempts to identify a genetic defect suggest that CVID is rather a complex disease with defects in several genetic loci. Grimbacher et al²² found 4 individuals with homozygous mutations in *ICOS*. B cells from these patients were reduced, whereas T cells were normal. Thus, in this elusive condition, *ICOS* became the first gene in which a mutation could be identified. Dadi et al²³ added a mutation in the *CD3 δ* unit to the list of genes that cause T-cell deficiency by using microarray techniques to identify the absence of gene expression in thymus tissue of 3 patients with severe combined immunodeficiency (SCID) and an unknown genetic defect. Remarkably, this defect caused severe immunodeficiency compared with the milder clinical consequences of mutated CD3 γ , a finding that underscores the role of the different components of the CD3 protein complex. Homozygous mutations in the uracil N-glycosidase (UNG) gene were reported to cause autosomal recessive hyper-IgM, which may also result from mutations in the activation-induced adenosine deaminase and in the CD40 genes. Three patients with UNG deficiency had defective class-switch recombination with mild defect of somatic hypermutation.²⁴ This report supports the hypothesis that activation-induced adenosine deaminase deaminates cytosines in targeted DNA, followed by uracil removal.

Genetic defects in innate immunity were also described in 2003. By using linkage analysis, Hernandez et al²⁵ localized the gene associated with WHIM (warts,

hypogammaglobulinemia, and myelokathexis) to a 12-cM region on chromosome 2q21. A candidate gene in that region, the chemokine receptor CXCR4, was sequenced, and mutations causing truncations in this protein were found. Fieschi et al²⁶ reported 41 patients with complete IL-12R β 1 deficiency from 29 unrelated kindreds. Unexpectedly, these patients had good prognoses. The only opportunistic infections observed were salmonellosis and mycobacterial infections. A defect in the type I IFN pathway was described for the first time in 2 unrelated infants with severe susceptibility to mycobacterial infections and viral diseases.²⁷ Mutations in the transcription factor signal transducer and activator gene were found in these infants. EBV-transformed cells from these patients were unable to be activated by IFN- α . The same group described another deficiency of innate immunity in 3 unrelated children who presented with recurrent pyogenic infections early in life, but these infections became uncommon as the children grew older.²⁸ After ruling out known immunodeficiencies, the authors found that the patients' monocytes lacked cytokine responses to LPS. This finding turned their attention to TLR pathways. Cells from these patients did not respond to ligands for TLR1 to TLR6 and TLR9. Mutations were not found in the transcription factors *MYD88*, *TIRAP*, *IRAK1*, *IRAK2*, and *IRAKM*. However, mutations were found in *IRAK4*. Northern blots were not able to detect mRNA for this gene. The susceptibility of *IRAK4*-deficient patients to pyogenic bacteria contrasts with mouse models with deficiencies in TLR pathways that are susceptible not only to pyogenic bacteria but also to a variety of infections.

Intravenous Igs

The mechanisms of action of Igs in the suppression of inflammatory diseases are not well defined, and many different hypotheses have been proposed. Altznauer et al²⁹ provided an explanation for previously described inhibitory and stimulatory effects of intravenous Ig (IVIG) on Fas-mediated apoptosis of neutrophils. They showed that IVIG at low dose (1-10 mg/mL) was able to inhibit apoptosis induced by anti-CD95 antibodies, but at higher concentrations (10-50 mg/mL), this inhibition disappeared, and apoptosis increased. The authors concluded that other mechanisms of action different from Fas-mediated apoptosis might play a role in explaining these effects. A note of caution in the use of Igs in patients with cardiovascular disease was presented in a letter to the Journal by Brown and Ballas.³⁰ They reported 3 patients with a diagnosis of CVID (without autoimmune disease) who developed acute thromboembolic events after they received IVIG at replacement doses. Two of the patients had coronary artery disease, and 1 had carotid artery stenosis. This report underscores the importance of a complete evaluation of patients, particularly patients with cardiovascular disease, who may benefit from iso-osmolar IVIG preparations and a slower infusion rate, as suggested by the authors.

Gene therapy for SCID

Shortly after it was reported that gene therapy for X-linked SCID (XSCID) was successfully achieved in 4 of 5 infants treated,³¹ 1 of the patients was diagnosed with leukemia, 3 years after he received gene therapy.³² A second child treated with gene therapy for XSCID showed evidence of leukemia a few months later. The investigators showed that these patients had developed clonal proliferations of T cells as a result of a retroviral vector insertion in the oncogene *LMO2*, which was aberrantly expressed.³³ Both children were the youngest to receive the gene-corrected cells, younger than 3 months. They were treated with chemotherapy, and the first patient subsequently received a successful bone marrow transplantation. Both patients are now in remission and well. This unfortunate adverse event suggests that, under certain circumstances, gene therapy may induce uncontrolled cell proliferation at higher frequencies than previously estimated. None of the other patients who received gene therapy has had a similar adverse effect. Although these occurrences provided a cautionary note in the field, soon after, gene therapy was demonstrated to be successful in another type of SCID caused by mutations in the adenosine deaminase (ADA) gene.³⁴ Attempts to immunoreconstitute ADA-SCID patients with gene therapy started in 1990, but it was only with the use of better transduction techniques for hematopoietic stem cells and the use of a conditioning regimen that gene therapy was proven successful.

Mechanisms of allergy

Eosinophilic bronchitis is a condition similar to asthma presenting with corticosteroid-responsive cough and airway inflammation but without airway hyperreactivity. Brightling et al³⁵ sought to test whether the mechanism of inflammation was mediated by T_H2 cytokines. They studied bronchial mucosal biopsies of 16 patients with eosinophilic bronchitis, 12 patients with asthma, and 14 normal controls. T cells expressing IL-4 and IL-5 were similarly increased in asthmatic patients and in patients with eosinophilic bronchitis compared with controls. This study suggests that increased T_H2 cytokines are not sufficient to explain the pathophysiology of asthma, particularly the airway hyperreactivity.

The inverse relationship between early exposure to pathogens and the development of allergy, the hygiene hypothesis, has been the subject of several studies recently. An opportunity to test this hypothesis was presented to Marks et al,³⁶ who studied 309 children between 7 and 14 years who were BCG-vaccinated and 442 children who were not BCG-vaccinated. More vaccinated children attended child care before age 2 years (29.6% vs 20.3%). Supporting the hygiene hypothesis, authors found that of children with a family history of asthma, children who had been vaccinated with BCG were less likely to develop asthma than children who had not.

New developments in immunotherapy

The mechanisms for the T_H2 response to T_H1 response switch during specific immunotherapy with allergens are not well defined. The signaling lymphocyte activation molecule (SLAM), transcription factor T-box expressed in T cells (T-bet), and IL-12 receptor are markers of T_H1 response that were investigated in patients who received immunotherapy.³⁷ Thirty patients allergic to pollen receiving immunotherapy were studied before immunotherapy and after 1 year on therapy, along with 10 patients with allergy not receiving therapy and 10 normal controls. After 1 year, significant increases were found in SLAM expression of lymphocytes stimulated *in vitro* with timothy or birch pollen extracts, which correlated with symptom improvement. However, those with the least improvement had the highest SLAM expression. Samples obtained during the maintenance phase were available from 10 patients. They found that symptom improvement was associated with early and transient increase of SLAM. Early SLAM expression may become a marker of response to immunotherapy.

Specific oligopeptides are attractive for immunotherapy because they are able to participate in T-cell antigen presentation, and these linear peptides lack the conformation necessary to bind the antigen site of IgE antibodies and elicit an anaphylaxis reaction. Sixteen patients sensitive to bee venom were enrolled in an immunotherapy protocol using 3 peptides covering the 134-amino acid sequence of phospholipase A2 (PLA2).³⁸ Patients were injected weekly for 7 weeks with 100 μ g PLA2 or albumin. The peptide injections were shown to be well tolerated. The patients developed T-cell responses and reduced skin test reactivity to PLA2, demonstrating the safety and efficacy of these linear peptides.

Fiset et al³⁹ developed an antisense oligonucleotide to inhibit IL-4 synthesis and thus decrease T_H2 -mediated inflammation. Nasal mucosa biopsies from 7 patients with ragweed allergy and undergoing sinus surgery were cultured *in vitro* with or without the IL-4 antisense oligonucleotide, and with sense nucleotide as a control. The nasal mucosa culture was then exposed to ragweed allergen for 1 day. IL-4 mRNA-bearing cells were significantly reduced in cultures exposed to IL-4 antisense compared with cultures that received ragweed alone or ragweed with sense oligonucleotide. IFN- γ mRNA-bearing cells were increased in those treated with the antisense oligonucleotide. Mucosal eotaxin and IgE levels were reduced. This study demonstrated the potential of oligonucleotides for therapeutic use in allergic rhinitis and asthma.

T-cell immunity and Antarctic winter

The isolation and confinement of explorers in the Antarctic winter affect the immune system, in particular cytokine levels. This extreme environment constitutes a good model for the effects of space flights in human beings. Shirai et al⁴⁰ studied peripheral blood mononuclear cells from 40 normal subjects before, during, and

after a prolonged winter stay in an Antarctic facility. Levels of all cytokines studied—IL-1, IL-1Ra, IL-2, IL-6, IL-10, and TNF- α —decreased and remained low. Other findings were higher percentages of NK cells, lower IgE levels, and increased T_H1:T_H2 ratios. These observations are pertinent to the US Space Program, which has received renewed government support for space exploration.

HIV infection

Differences in HIV transmission have been attributed in part to mucosal factors. One of these factors is the antimicrobial peptide group called *defensins*, which are synthesized in high levels at the mucosa. Quinones-Mateu et al⁴¹ reported that HIV-1 particles induce the expression of human β -defensins (hBDs) in oral epithelial cells. hBDs are small cationic molecules of approximately 35 to 45 amino acid residues. HIV-1 particles were shown bound to hBD by electron microscopy. A related defensin molecule called *retrocyclin* is an octadecapeptide that protects lymphocytes from HIV-1 infection *in vitro* by binding to gp120.⁴²

Research on the origins of HIV-1 had a breakthrough with a report demonstrating with phylogenetic studies that simian immunodeficiency virus strains from recapped mangabeys and from greater spot-nosed monkeys recombined in the chimpanzee to form simian immunodeficiency virus cpz (which has been shown to be most closely related to HIV-1).⁴³ This study provides evidence of a nonhuman species that had acquired a lentivirus by cross-species recombination transmission under natural conditions. Another advance in the molecular biology of HIV was the evidence for an *in vivo* role of the viral protein Nef. It is known that Nef downregulates CD4 expression and interferes with NF κ B pathway activation. Casartelli et al⁴⁴ studied 182 HIV Nef variants from 6 HIV non-progressor, 4 rapid progressor, and 3 slow progressor children, and showed a relationship of number of Nef mutations with progression of disease. The 6 non-progressors had more mutations in the Nef protein core domain.

Some of the immunologic characteristics of long-term progressors were partially reproduced in a series of 6 HIV-1-infected patients receiving structured treatment interruption and early treatment during acute HIV infection.⁴⁵ Both groups had in common the expansion and maintenance of HIV-1-specific CD8⁺ T cells, adding evidence for the potential of immune control of HIV infection. Differences in the immunologic response of HIV-1-infected children and adults were shown by Feeney et al,⁴⁶ who reported that HIV infected children who controlled viremia without antiretroviral therapy had the largest anti-HIV-1 Gag-specific CTL response. Fourteen of 17 patients with low viremia had a strong anti-HIV-1 p24 CTL response, in contrast with only 1 of 9 who had elevated viremia. A more frequent and stronger specific CTL response was found in children compared with adults with similar viral loads. Scott et al⁴⁷ found that anti-HIV-1 Gag-specific CD4⁺ T cells are less frequent when HIV-infected children have less than 50 viral copies/

mL plasma than when children have active viral replication. These studies underscore the need of vaccines to synergize with antiretroviral therapy to conserve the antiviral immune response in children.

Mitochondrial toxicity induced by zidovudine was studied measuring the ratio of mitochondrial DNA to nuclear DNA in 30 infants (1-2 years of age) from HIV-infected mothers and 20 controls, 10 of whom received zidovudine prophylaxis.⁴⁸ There was statistically significant decreased ratio in the HIV-infected group that received prophylaxis compared with normal infants who did not receive zidovudine. However, it is not certain how this difference translates into subsequent clinical symptoms and illness.

HIV decay kinetics studies have helped to estimate the life expectancy of HIV in patients receiving antiretroviral therapy. Siliciano et al⁴⁹ reported new estimates after analyzing samples from 62 patients on highly active antiretroviral therapy for as long as 7 years. The authors reported a very slow rate of HIV-1 decay in plasma, with a t_{1/2} of 44.2 months, indicating that it would take 51 years to clear a reservoir of only 1 million HIV-1-infected cells. HIV eradication with current anti-HIV drugs may not be achieved during the lifetime of the patient.

Kilby and Eron⁵⁰ reviewed recent developments in anti-HIV drugs that are based on cell entry. The Federal Drug Administration has approved T-20, or Enfuvirtide, which inhibits the fusion step between HIV-1 gp41 and CD4. Enfuvirtide is effective only in combination with other antiretroviral drugs because of development of drug resistance. Other groups of cell entry inhibitors are the chemokine receptor inhibitors and the nonspecific inhibitors of attachment, which are proposed to be used as topical microbicides. The last 2 groups are being evaluated for side effects and viral resistance mechanisms. In phase III studies, 504 HIV-infected patients who failed to respond to current treatment and had viral copies of at least 5000 copies/mL from Europe and Australia were randomized to receive Enfuvirtide or placebo in addition to antiretroviral drugs and were followed for as long as 24 weeks.⁵¹ There was reduction in viral load in 42.7% of the patients in the Enfuvirtide group compared with 20.7% in the control group. An increase of CD4⁺ T-cell counts was also detectable in the Enfuvirtide group (mean, 65.5 cells/mm³) compared with the control group (mean, 38.0 cells/mm³). In a similar randomized, open-label trial in North and South America, patients receiving Enfuvirtide in addition to optimized antiretroviral therapy had greater reduction of viral load and higher CD4⁺ T-cell counts than patients receiving antiretroviral therapy alone.⁵³

An elegant study by Kawai et al⁵² described a polyphenol compound obtained from green tea that is able to bind specifically to CD4 molecules, but not other cell surface molecules, and competitively inhibit HIV-1 gp120 binding. These properties were further investigated by molecule modeling.⁵⁴ More research is needed to determine the possibility of achieving effective concentrations *in vivo* and possible negative consequences in T-cell function.

CONCLUSION

Every year witnesses outstanding advances in the immunologic sciences either in basic research, which ultimately translates into clinical applications, or in clinical research itself. In 2003, the importance of innate immunity in the control of infections was again demonstrated by the interactions of TLRs and adaptive immunity (Table I). The role of CD25⁺CD4⁺ regulatory cells and the possible role of dendritic cells in the development of tolerance and allergic responses are actively investigated. In clinical immunology, new genetic defects responsible for previously obscure immunodeficiency syndromes continue to increase our knowledge of the intricate mechanisms of immunity (Table II). Advances in genetics have made it possible to cure XSCID by means of gene therapy and have allowed the prompt diagnosis and treatment of the complications of this new modality. Continuing research into the mechanisms of allergy suggests that modification of whole-extract immunotherapy with peptide immunotherapy may avoid the risk of anaphylaxis. Advances in HIV biology are helping the development of new antiviral treatments that provide hope for the eventual eradication of this virus. Finally, research on the effects of extreme environmental conditions on the immune system may prepare human beings for safer space flights.

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