vivo with proteins that exhibit not only tyrosine but also serine and threonine kinase activities (*23*). Thus, Akt and other related proteins may represent a functional link between tyrosine and serine-threonine phosphorylation pathways.

The oncogenic activation of the retrovirally transduced *Akt* may be due to its fusion to *gag*. Because of the presumed myristylation of its NH_2-terminal Gly[1] residue (*25*), Akt is expected to be anchored to the cell membrane where its SH2-like domain might interact with tyrosine kinases and other phosphotyrosine-containing targets.

Note added in proof: A gene, *rac*, has been cloned that encodes a protein kinase related to PKC and the cyclic adenosine monophosphate–dependent protein kinase. The gene appears to be the human homolog of c-*akt* (*25a*).

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Defining Protective Responses to Pathogens: Cytokine Profiles in Leprosy Lesions

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The immunological mechanisms required to engender resistance have been defined in few infectious diseases of man, and the role of specific cytokines is unclear. Leprosy presents clinically as a spectrum in which resistance correlates with cell-mediated immunity to the pathogen. To assess in situ cytokine patterns, messenger RNA extracted from leprosy skin biopsy specimens was amplified by the polymerase chain reaction with 14 cytokine-specific primers. In lesions of the resistant form of the disease, messenger RNAs coding for interleukin-2 and interferon-γ were most evident. In contrast, messenger RNAs for interleukin-4, interleukin-5, and interleukin-10 predominated in the multibacillary form. Thus, resistance and susceptibility were correlated with distinct patterns of cytokine production.

ONE OF THE KEY FUNCTIONAL PArameters determining the outcome of immune responses to infectious agents is the nature of the cytokines produced locally by immune cells, yet the patterns of cytokine production are unknown for most infectious diseases of man. Leprosy offers an attractive model for investigating the role of cytokines in resistance or susceptibility to infection for several reasons. Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that primarily affects skin, the lesions of which are readily accessible to cellular and molecular analysis. Because leprosy is generally not life-threatening, the dynamics of infiltrating cells can be investigated over time. It is a disease that presents as a clinical and immunologic spectrum (*1*). $CD4^+$ T cells predominate in tuberculoid (resistant) lesions; in contrast $CD8^+$ T phenotypes predominate in lepromatous (susceptible) lesions (*2*). $CD4^+$ cells from tuberculoid lesions, but not lepromatous ones, proliferate in culture in response to *M. leprae* (*3*). On the other hand, $CD8^+$ cells from lepromatous lesions, but not tuberculoid ones, can be activated specifically by *M. leprae* to suppress CD4 T cell proliferation in vitro (*4*).

We examined the patterns of cytokine expression in lesions from 16 individuals (eight lepromatous and eight tuberculoid) who were at the poles of the spectrum of leprosy, to identify correlates of resistance and susceptibility to the pathogen (Fig. 1). Because several cytokine mRNAs exist at low levels in biopsy samples, we extracted total RNA from biopsy specimens, reverse-transcribed the polyadenylated mRNAs to obtain lymphokine cDNAs, and detected those cDNAs with high sensitivity by the polymerase chain reaction (PCR) with specific cytokine primers (*5*). PCR is a semiquantitative technique at best; to provide meaningful comparisons between different individuals and forms of the disease, we normalized the cDNAs to the β-actin PCR product, a marker for all cells, and to the

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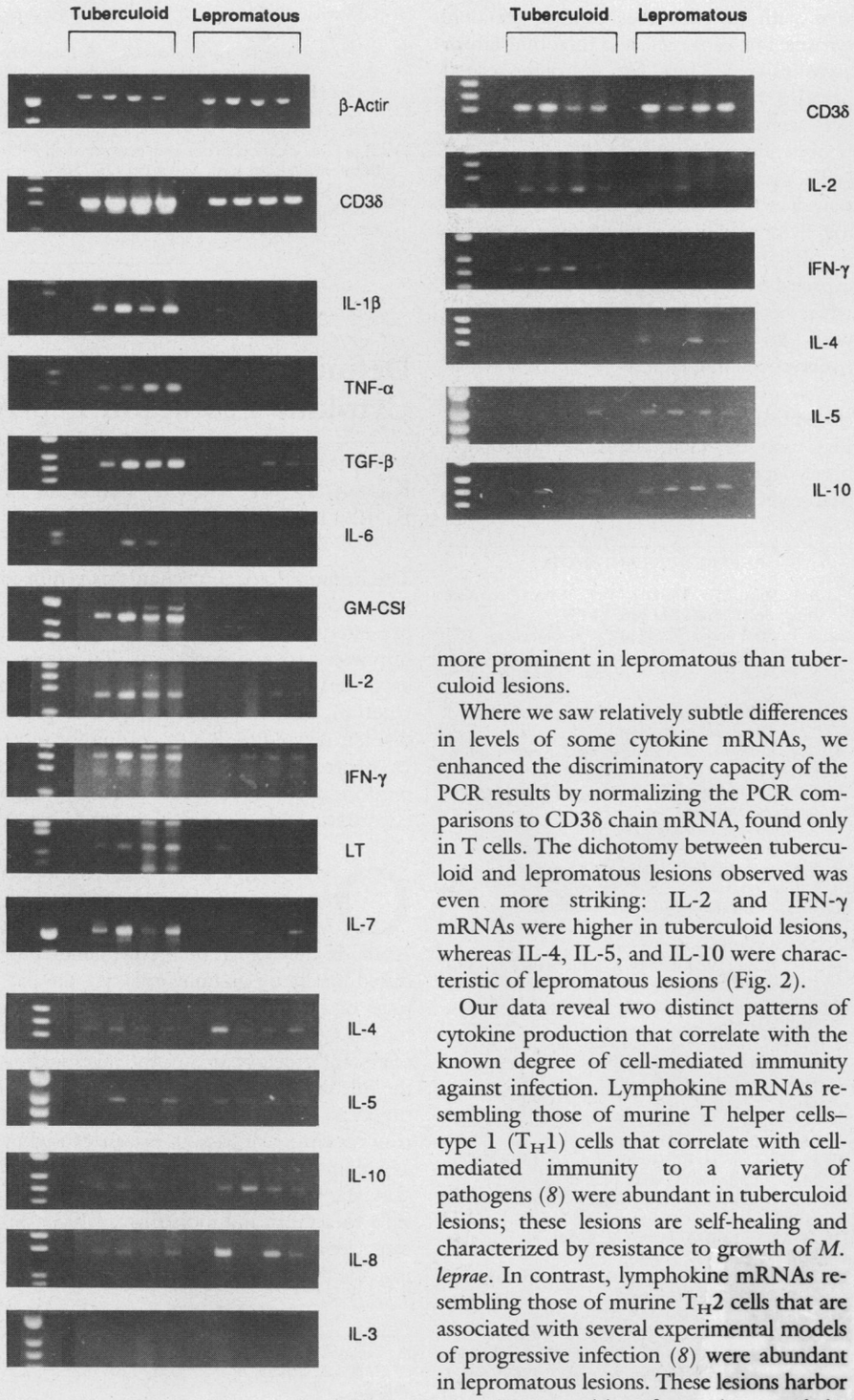

Fig. 1. Cytokine mRNAs in leprosy lesions revealed by PCR normalized to β-actin. The data shown are for a representative 8 out of 16 individuals studied. Individuals with leprosy were classified into tuberculoid and lepromatous according to the clinicopathologic criteria of Ridley (*1*). Skin biopsy specimens (obtained with informed consent at the time of diagnosis) were obtained by punch or scalpel technique, embedded in OCT medium (Ames, Elkhart, Indiana), and snap frozen in liquid N_2. The majority of the cellular infiltrate in these lesions is composed of granulomas that comprise lymphocytes and macrophages. Immunostaining has revealed that 53% of the infiltrating cells in tuberculoid lesions and 40% in lepromatous lesions are T cells; the rest are macrophages (*13*). Forty 5-μm sections from each skin biopsy specimen were placed in 0.5 ml of 4 M guanidinium isothiocyanate buffer. RNA was isolated by CsCl gradient. The cDNA was synthesized with avian myeloblastosis virus reverse transcriptase (Bethesda Research Laboratories) and poly(dT) (Pharmacia) priming and used for PCR amplification. Equivalent quantities of total RNA and cDNA were obtained from similar volumes of tuberculoid and lepromatous tissue. Samples were amplified by PCR with Taq polymerase (Promega Biotec) by 35 or 40 cycles of denaturation at 94°C for 1 min and annealing-extension at 55°C (for IL-2, IL-7, and IFN-γ) or 65°C for 2 min. PCR product was analyzed by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining. We verified these PCR products by probing samples transferred to nylon membrane with a ^{32}P-labeled internal oligonucleotide. Sensitivity of PCR was equivalent for each cytokine based on titration of cytokine plasmid cDNAs, was 10^2 to 10^3 copies for each cytokine, and was proportional to the number of copies of plasmid cDNA to at least 10^8 copies. Equivalent efficiency of amplification was observed in the absence and presence of an irrelevant cDNA derived from normal skin. The cDNAs derived from lepromatous skin lesions were not inhibitory to the PCR reaction because addition of these cDNAs to tuberculoid lesion–derived cDNAs did not diminish the product. The β-actin PCR product of tuberculoid and lepromatous lesions was of similar magnitude, and PCR amplification for several cytokine mRNAs (IL-4, IL-5, and IL-10) from lepromatous lesions yielded significant product. Although the PCR products were not quantitated, the results show striking differences in the intensity of PCR products for a given cytokine mRNA in one extreme of leprosy versus the other, representing 10- to 100-fold differences.

CD3δ PCR product, a marker of T cells.

Cytokine mRNA profiles of tuberculoid lesions differed from lepromatous lesions, and those differences were consistent across lesions of all patients. Cytokine mRNAs predominantly produced by macrophages [interleukin-1β (IL-1β), tumor necrosis factor–α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factor–β1 (TGF-β1), and IL-6] were more abundant in the tuberculoid than in the lepromatous lesions studied. Lymphokines produced mainly by T cells [IL-2, interferon-γ (IFN-γ), and lymphotoxin (LT)] were abundant in tuberculoid lesions but virtually absent in lepromatous lesions. This confirms data from immunohistochemistry and in situ hybridization that the percentage of cells producing IL-2 (*6*) and IFN-γ (*7*) was higher in tuberculoid lesions than lepromatous lesions. In contrast, IL-4 and IL-10 mRNAs appeared more prominent in lepromatous than tuberculoid lesions.

Where we saw relatively subtle differences in levels of some cytokine mRNAs, we enhanced the discriminatory capacity of the PCR results by normalizing the PCR comparisons to CD3δ chain mRNA, found only in T cells. The dichotomy between tuberculoid and lepromatous lesions observed was even more striking: IL-2 and IFN-γ mRNAs were higher in tuberculoid lesions, whereas IL-4, IL-5, and IL-10 were characteristic of lepromatous lesions (Fig. 2).

Our data reveal two distinct patterns of cytokine production that correlate with the known degree of cell-mediated immunity against infection. Lymphokine mRNAs resembling those of murine T helper cells–type 1 (T_H1) cells that correlate with cell-mediated immunity to a variety of pathogens (*8*) were abundant in tuberculoid lesions; these lesions are self-healing and characterized by resistance to growth of *M. leprae*. In contrast, lymphokine mRNAs resembling those of murine T_H2 cells that are associated with several experimental models of progressive infection (*8*) were abundant in lepromatous lesions. These lesions harbor enormous quantities of organisms, and the affected individuals are immunologically unresponsive to *M. leprae* antigens. The PCR technique does not enable us to distinguish which cells are producing the mRNAs; there are, however, twice as many $CD4^+$ as $CD8^+$ cells in tuberculoid lesions, and conversely, twice as many $CD8^+$ cells as $CD4^+$ cells in lepromatous lesions (*2*). Because *M. leprae*–reactive T cell clones from leprosy patients across the spectrum produce patterns of lymphokines in vitro with remarkable simi-

larity to the profiles described here in lesions (*9*), we believe the lymphokine mRNAs in lesions reflect the pattern of T cell subsets associated with resistant and susceptible forms of the disease.

Studies of immune responses to leishmaniasis in BALB/c mice have demonstrated that $CD4^+$ T cells capable of adoptively transferring resistance to infection produce predominantly IL-2 and IFN-γ in vitro, whereas those that induce exacerbation and more rapid lesion formation produce primarily IL-4 and IL-5 (*10*). Treatment of susceptible mice with antibodies to IL-4 resulted in increased IFN-γ production with a concomitant increase in the host resistance to the *Leishmania major* parasites (*11*). Although we cannot differentiate whether the cytokine patterns in the leprosy lesions are the cause or result of disease, the extensive studies in murine models suggest that the cytokine patterns are critical to the development of the characteristic lesions in leprosy. Such cytokine patterns are likely to contribute to the pathogenesis of other chronic infectious diseases of man. Further long-term horizontal studies of affected individuals with and without treatment by immunotherapy should define the dynamics of cytokine patterns in the pathogenesis of human infections.

Developing new and improved vaccines (*12*) requires greater understanding of the complex immunoregulatory mechanisms that determine protective immunity, unresponsiveness, and tissue damage. The patterns of lymphokines and cytokines produced at the sites of infection, together with specific cell-mediated cytotoxic activities, are most likely to determine the outcome of the host-pathogen interaction. Because several of the cytokines found particularly in self-healing lesions can produce tissue damage, as well as enhanced microbicidal activity (such as TNF-α, IFN-γ, and LT), it is appropriate to wonder about the extent to which the pathogenesis of these infectious diseases, such as the destruction of the nerves in leprosy or of the lungs in tuberculosis, is the price to be paid by the host for protection.

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Differing Lymphokine Profiles of Functional Subsets of Human CD4 and CD8 T Cell Clones

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Functional subsets of human T cells were delineated by analyzing patterns of lymphokines produced by clones from individuals with leprosy and by T cell clones of known function. CD4 clones from individuals with strong cell-mediated immunity produced predominantly interferon-γ, whereas those clones that enhanced antibody formation produced interleukin-4. CD8 cytotoxic T cells secreted interferon-γ. Interleukin-4 was produced by CD8 T suppressor clones from immunologically unresponsive individuals with leprosy and was found to be necessary for suppression in vitro. Both the classic reciprocal relation between antibody formation and cell-mediated immunity and resistance or susceptibility to certain infections may be explained by T cell subsets differing in patterns of lymphokine production.

T CELLS HAVE BEEN DIVIDED INTO subsets based on expression of different surface antigens. $CD4^+$ T cells function as helper cells in antibody formation and mediate delayed-type hypersensitivity (DTH), a form of cell-mediated immunity. $CD8^+$ T cells encompass cytotoxic T lymphocytes (CTLs) and antigen-specific T suppressor (T_S) cells. Murine $CD4^+$ T cells effecting different immunological functions have been further subdivided according to distinct, nonoverlapping patterns of lymphokine production (*1*). $CD4^+$ T cells involved in DTH responses (designated T_H1 cells) produce interleukin-2 (IL-2), interferon-γ (IFN-γ), lymphotoxin, and other lymphokines but little IL-4 or IL-5. Reciprocally, murine $CD4^+$ T helper cell clones that regulate antibody production (T_H2 cells) preferentially produce IL-4, IL-5, and IL-6 but little IL-2 or IFN-γ. However, most human $CD4^+$ clones derived from healthy donors produce IL-2, IFN-γ, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), or combinations thereof that have been difficult to relate to the murine T_H1 and T_H2 subsets or to specific biological function (*2*). Human T

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