Dörken and G. Moldenhauer for providing MAbs, and Dr. H. Messner for providing tumor cells. We also thank D. Scheppelmann and H.-P. Meinzer for the computerized image analysis of normal and apoptotic cells. Supported by grants from the Bundesministerium für Forschung und Technologie, Bonn, the Ministerium für Wissenschaft und Kunst, Stuttgart, and the Tumor Center Heidelberg/Mannheim, FRG. A.M.J.P. was supported by the Boehringer Ingelheim Fonds, Stuttgart, FRG.

3 February 1989; accepted 19 May 1989

The Reservoir for HIV-1 in Human Peripheral Blood Is a T Cell That Maintains Expression of CD4

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Human immunodeficiency virus type 1 (HIV-1) selectively infects cells expressing the CD4 molecule, resulting in substantial quantitative and qualitative defects in $CD4^+$ T lymphocyte function in patients with acquired immunodeficiency syndrome (AIDS). However, only a very small number of cells in the peripheral blood of HIV-1–infected individuals are expressing virus at any given time. Previous studies have demonstrated that in vitro infection of $CD4^+$ T cells with HIV-1 results in downregulation of CD4 expression such that CD4 protein is no longer detectable on the surface of the infected cells. In the present study, highly purified subpopulations of peripheral blood mononuclear cells (PBMCs) from AIDS patients were obtained and purified by fluorescence-automated cell sorting. They were examined with the methodologies of virus isolation by limiting dilution analysis, in situ hybridization, immunofluorescence, and gene amplification. Within PBMCs, HIV-1 was expressed in vivo predominantly in the T cell subpopulation which, in contrast to the in vitro observations, continued to express CD4. The precursor frequency of these HIV-1–expressing cells was about 1/1000 $CD4^+$ T cells. The $CD4^+$ T cell population contained HIV-1 DNA in all HIV-1–infected individuals studied and the frequency in AIDS patients was at least 1/100 cells. This high level of infection may be the primary cause for the progressive decline in number and function of $CD4^+$ T cells in patients with AIDS.

THE HUMAN IMMUNODEFICIENCY virus type 1 (HIV-1), the etiologic agent of the acquired immunodeficiency syndrome (AIDS), selectively infects cells expressing the CD4 molecule, including T lymphocytes and cells of the monocyte/macrophage lineage (*1*). In vitro infection of cells with HIV-1 results in a decreased expression of the CD4 molecule on the surface of the infected cells (*2*).

Patients with AIDS have severe depression of the normal cell-mediated immune mechanisms that is partially attributed to the considerable depletion of CD4 lymphocytes (*3*). Despite this, examination of cells from lymph nodes and peripheral blood from patients with AIDS and AIDS-related complex (ARC) has revealed a very low frequency of viral RNA synthesis, generally occurring in 1/100,000 to 1/10,000 of total mononuclear cells (*4*). However, it is possible that a larger proportion of cells may be latently infected (containing proviral DNA but not expressing viral mRNA or protein). Until the development of gene amplification [polymerase chain reaction (PCR)] methodology (*5, 6*), HIV-1–infected cells not expressing virus were not readily detectable by available techniques.

In the present study, blood was obtained from HIV-1 culture-positive patients with AIDS either directly in heparinized syringes or via apheresis and subjected to Ficoll-Hypaque separation (*7*). First, peripheral blood mononuclear cells (PBMCs) from patients were stained with fluorescein isothiocyanate (FITC)–conjugated antibody to CD3 and sorted by a fluorescence-activated cell sorter (FACS) into $CD3^+$ and $CD3^-$ populations. Sorted cells were cocultivated with an excess of normal phytohemagglutinin (PHA)-stimulated blast cells and we determined the time to peak viral expression, a highly consistent and reproducible parameter of viral expression. A predominance of HIV-1 expression in the >98% enriched $CD3^+$ population, as determined by the time to peak syncytia formation (Fig. 1A) and reverse transcriptase (RT) activity (Fig. 1B), was seen. Similar results were obtained in seven additional AIDS patients. Delayed expression of HIV-1 in cells that were initially 99% $CD3^-$ cells (Fig. 1B) was due to outgrowth of the few contaminating $CD3^+$ cells. Phenotypic analysis of noncocultivated enriched $CD3^-$ cells grown under the same conditions revealed that 35 to 65% of the cells were $CD3^+$ by day 10 in culture.

In the second series of experiments, PBMCs from AIDS patients were double-stained with FITC-conjugated anti-CD3 and anti-CD4 and sorted by FACS into $CD3^+/CD4^+$ and $CD3^+/CD4^-$ populations. These sorted cells were cocultivated with an excess of normal PHA-stimulated blast cells and showed a predominance of HIV-1 expression in the highly enriched (98 to 99%) $CD4^+$ T cell population as determined by the time to peak syncytia formation (Fig. 1C) and RT activity (Fig. 1D). Similar results were obtained in seven additional AIDS patients. The phenotypic analysis of freshly sorted $CD3^+/CD4^+$ cells revealed a greater than 98 to 99% $CD4^+$ purity in most experiments when stained with the monoclonal antibody to Leu 3a. Again, the delayed expression of HIV-1 in cells that were initially 99% $CD4^-$ (Fig. 1D) was most likely due to outgrowth of a few contaminating $CD4^+$ T cells. Phenotypic analysis of non-cocultured enriched $CD4^-$ T cells grown under the same conditions revealed that 30 to 55% of the cells were $CD4^+$ by day 10 in culture.

In situ hybridization for HIV-1 viral RNA was then performed at time zero on the highly enriched $CD3^+/CD4^+$- and $CD3^+/CD4^-$-sorted PBMCs. There was a predominance of viral expression in the $CD4^+$ T cell population at a frequency of about 1/1000 cells in four AIDS patients ($\bar{X}$ ± SEM per 1000 cells was 0.95 ± 0.21) (Fig. 2A). This is in comparison to a level of viral expression in the $CD4^-$ T cell population of <1/100,000 cells (Fig. 2B), which is equivalent to background signal in controls. The frequency of in situ–positive $CD4^+$ T cells remained unchanged in three of the patients reexamined at 6 to 12 months after the initial studies.

Indirect immunofluorescence studies for HIV-1 viral antigens was also performed at time zero on highly enriched $CD3^+/CD4^+$- and $CD3^+/CD4^-$-sorted PBMCs. These demonstrate a predominance of viral expression in the $CD4^+$ T cell population at a frequency of about 1/1000 cells in four AIDS patients ($\bar{X}$ ± SEM per 1000 cells was 1.10 ± 0.35 (Fig. 2C). This is in comparison to a level of viral expression in the $CD4^-$ T cell population of <1/10,000 cells

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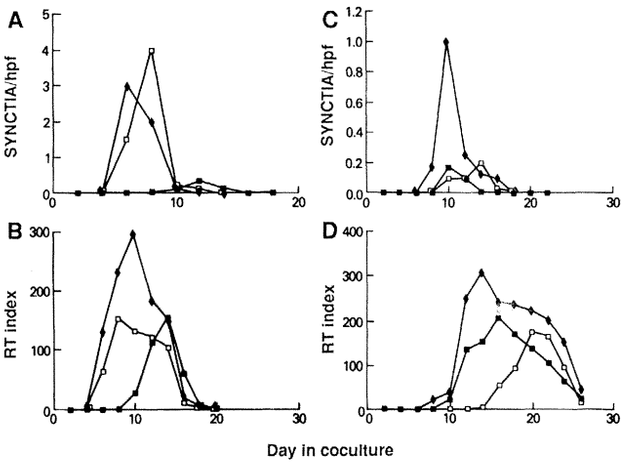

Fig. 1. Expression of HIV-1 in highly enriched $CD3^+$ cells (□, unfractionated; ◆, $CD3^+$; ■, $CD3^-$) as measured by syncytia (**A**) and RT activity (**B**) and in sorted and highly enriched $CD3^+/CD4^+$ cells (■, unfractionated; ◆, $CD3^+/CD4^+$; □, $CD3^+/CD4^-$) as measured by syncytia (**C**) and RT activity (**D**). Cells were isolated from AIDS patients and cocultivated with normal PHA-stimulated blast cells. RT activity is expressed as RT index, which is the RT activity in the sample divided by the RT activity in the negative control. For these experiments, PBMCs were stained with FITC-conjugated monoclonal antibodies to CD3 (anti-Leu4) or CD4 (anti-Leu3a) (Becton Dickinson Immunocytometry Systems), or both, determinants. The background controls consisted of PBMCs stained with a nonreactive monoclonal antibody isotype control. Fluorescence-labeled PBMCs were sorted with an EPICS C flow cytometer (Coulter Electronics) (*19*). The purity for all sorted cell populations used in these experiments exceeded 98 to 99% when analyzed by using parameters identical to those for sorting. Portions of both unfractionated and sorted cell populations (approximately 1.5 million cells) were cocultivated with an excess of normal PHA-stimulated blast cells, and every 2 days the cultures were examined for syncytia, supernatants were collected for RT activity (*20*), and the cultures were fed [10% fetal calf serum in RPMI 1640, 5% anti-α interferon (Interferon Sciences), and 10% purified interleukin-2 (Electronucleonics)]; hpf, high-powered field.

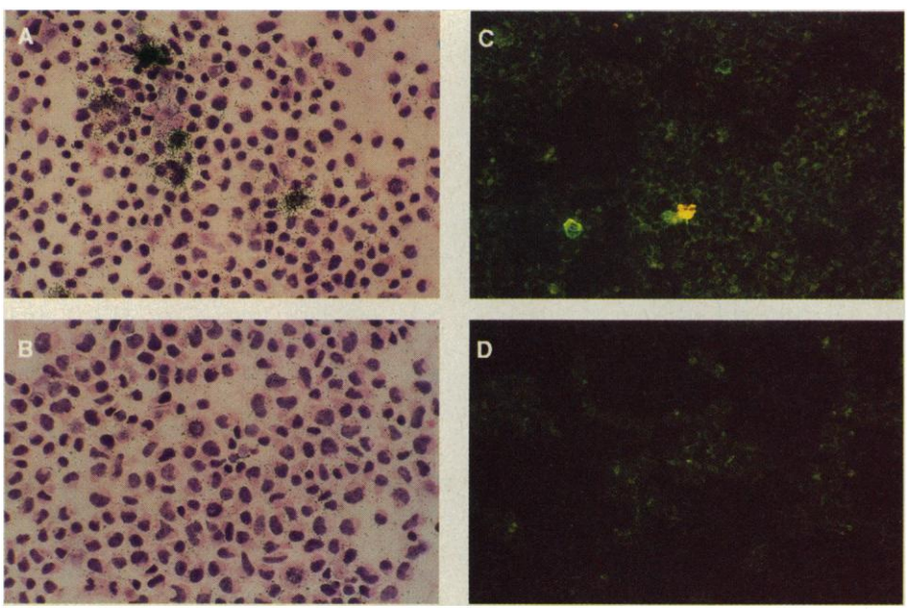

Fig. 2. HIV-1 RNA and protein in enriched populations. Highly enriched (>98 to 99%) $CD3^+/CD4^+$- and $CD3^+CD4^-$-sorted PBMCs derived from AIDS patients were examined at time zero by in situ hybridization (A) and (B), and by indirect immunofluorescence (C) and (D). For (A) and (B), approximately 100,000 cells were examined per patient; for (C) and (D), approximately 10,000 cells were examined per patient. (**A**) $CD3^+/CD4^+$ cells showing a cluster of HIV^+ cells. (**B**) $CD3^+/CD4^-$. For these experiments, FAC-sorted cells were centrifuged onto glass slides (siliconized). In situ hybridization of cells was performed by the general method described by Pardue (*21*). Modifications of the basic technique and detailed descriptions of these techniques have been published (*22*). PBMCs from AIDS patients that were sorted as $CD3^+/CD4^+$ and $CD3^+/CD4^-$ were examined at time zero by indirect immunofluorescence for expression of HIV-1 viral antigens. (**C**) $CD3^+/CD4^+$ cells; several fluorescent cells with viral expression in the $CD4^+$ T cell population. (**D**) $CD3^+/CD4^-$ cells. For these experiments, cells were cytocentrifuged, air-dried, and fixed in 50% acetone/50% methanol for 10 min at 4°C. Serum from a single AIDS patient or an HIV-1–negative volunteer was diluted in PBS (1:1000), added to the slides for 30 min, and removed by washing twice in PBS. FITC-conjugated goat anti-human antibody (1:500) (Jackson ImmunoResearch Laboratories) was added to the slides for 30 min in the dark followed by two additional washes in PBS. Cover slips were placed over a glycerol layer and the slides were examined for fluorescence-positive cells on a Nikon Labophot fluorescence microscope. All slides incubated with serum from an AIDS patient were compared with those incubated with normal serum to control for nonspecific binding. Observations in (A) and (C) are representative of findings made in four patients.

(Fig. 2D), which is equivalent to background immunofluorescence in controls.

To measure the frequency of HIV expression in the $CD3^+/CD4^+$ population of cells, limiting dilution analysis was done on these highly enriched, sorted PBMCs derived from AIDS patients cocultivated with normal PHA-stimulated blast cells. We determined precursor frequencies of HIV expressing $CD4^+$ T cells of 1/4000, 1/800, and 1/1200, respectively, in three AIDS patients. These frequencies were determined by Poisson distribution where 63% of RT-positive wells indicates one positive cell per well (*8*). These frequencies are in close agreement with those obtained by in situ hybridization and immunofluorescence.

To confirm our observations of the $CD4^+$ T cell as a reservoir for HIV-1 in the peripheral blood of patients with AIDS and to address the possibility that monocytes or other cells in the peripheral blood of AIDS patients may contain HIV-1 proviral DNA, we used the technique of gene amplification by means of PCR. First, PBMCs from four AIDS patients (representing advanced HIV infection) and ten healthy HIV-1 seropositive, culture-negative individuals (*9*) (representing very early HIV-1 infection) were sorted by FACS, resulting in populations with the following cell surface markers at purities of greater than 98 to 99%: $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, $CD14^+$ monocytes, and $CD19^+$ B lymphocytes. We then used PCR to determine the presence of HIV-1 DNA in the various enriched cell subpopulations. For each HIV-1–infected patient that was examined by PCR, we used three primer pairs, from the *env*, *gag*, and long terminal repeat (LTR) regions of the genome, respectively. HIV-1 DNA was detected in the enriched $CD4^+$ T lymphocyte (as well as in the unfractionated) population of cells in every patient examined by PCR (Fig. 3). In only 2 of 14 individuals was HIV-1 DNA detected in the enriched peripheral monocyte fraction by PCR. In no patient was HIV-1 DNA detected in either the $CD8^+$ T lymphocyte or $CD19^+$ B lymphocyte populations. As reciprocal controls for the sorted cells, the $CD4^-$ fraction (predominantly $CD8^+$ T cells) gave no signal, the $CD8^-$ fraction (predominantly $CD4^+$ T cells) gave a strong signal, and the $CD14^-$ population (containing some nonrosetted T cells) frequently gave a weak signal.

To determine the frequency of HIV-1

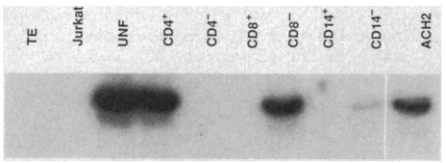

Fig. 3. PCR amplification of HIV-1 DNA in enriched populations. The oligomer hybridization autoradiograph demonstrates the presence of amplified HIV-1 DNA (with SK68/69 *env* primers, SK70 probe and 4-hour exposure) in the highly enriched (>98 to 99%) FAC-sorted $CD4^+$ T cell population as well as in the unfractionated PBMCs in a patient with AIDS (TE, buffer control; Jurkat, negative cell control; and ACH2, positive control). Similar results were obtained with *gag* (SK38/39) and LTR (SK29/30) primer pairs and their respective probes (*23*). For these experiments, some PBMCs from HIV-1–infected individuals were FITC-stained with monoclonal antibodies to either CD4 (anti-Leu-3a, T helper), or to CD8 (anti-Leu-2a, T suppressor) (Becton Dickinson). One half of the cells underwent enrichment for monocytes and B cells before cell sorting by T cell depletion with 2-aminoethylisothiouronium bromide–treated sheep red blood cells. The nonrosetted cells, which were rich in monocytes and B cells, were FITC-stained with monoclonal antibodies to CD14 (anti-Leu M3, monocyte) or to CD19 (anti-Leu-12, B cell) (Becton Dickinson). Fluorescence-labeled cells were then sorted as described (*19*). As reciprocal controls for the sorted cells, the $CD4^-$ population is predominantly $CD8^+$ T cells; the $CD8^-$ population is predominantly $CD4^+$ T cells; the $CD14^-$ population contains T cells, B cells, and natural killer (NK) cells; whereas the $CD19^-$ population contains T cells, monocytes, and NK cells. Cells to be examined for HIV-1 DNA were prepared, the DNA amplified, and oligomer hybridization carried out as described (*23*).

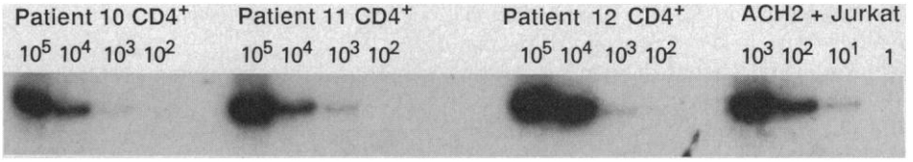

Fig. 4. The precursor frequency of $CD4^+$ T cells that contain HIV-1 DNA as detected by PCR. An oligomer hybridization autoradiograph (4-hour exposure) is shown. For these experiments, tenfold serial dilutions of the DNA lysates from 100,000 $CD4^+$ T cells obtained by FACS from three AIDS patients were each amplified for HIV-1 DNA with the SK68/69 *env* primers (and probed with SK70) as described (*23*). This was compared to the amplification of tenfold serial dilutions of DNA lysates of HIV-1–infected ACH2 cells, a chronically infected T cell clone that contains one proviral copy per cell. The amplification of the serial "standard" dilutions of ACH2 DNA reveals that the assay is capable of detecting ten HIV-1–infected ACH2 cells in the presence of excess control Jurkat cell DNA derived from 10^6 cells. Therefore, since we obtain a signal for 1000 $CD4^+$ T cells in each of the three AIDS patients equivalent to that obtained from ten infected ACH2 cells, one can deduce that at least 10/1000 or 1/100 $CD4^+$ T cells contain HIV-1 DNA. Similar results were obtained with *gag* (SK38/39) and LTR (SK29/30) primer pairs with their respective probes. This suggests that the ACH2 is homologous to many clinical HIV-1 isolates in these regions and can hybridize with these primers in a comparable manner.

DNA in $CD4^+$ cells, whether integrated DNA with or without mRNA expression, or unintegrated DNA, PCR was done on serial tenfold dilutions of the enriched $CD4^+$ T cell population from patients with AIDS. These amplifications were then compared with the PCR performed on serial dilutions of the ACH2 cell line, a chronically infected T cell line containing one DNA copy of HIV per cell (*10*). In the three patients with AIDS that were studied, we determined that at least 1/100 $CD4^+$ T cells contains HIV-1 DNA (Fig. 4). The possibility of multiple integrated or unintegrated copies of viral DNA must be considered. If only a very few peripheral blood cells contained large numbers of copies of viral DNA, there would have been disparate results when cells were serially diluted and then lysed for DNA studies versus when cells were lysed first and then serial dilutions of DNA were performed. However, the same results were obtained regardless of whether the cells were serially diluted before or after lysis for DNA. This suggests that the $CD4^+$ T cells from the patients contain predominantly one copy of viral DNA per cell.

The critical basis for the immunopathogenesis of HIV-1 infection is the depletion of the helper-inducer subset of T lymphocytes which express the CD4 molecule, resulting in profound immunosuppression (*3*). The $CD4^+$ T cell is the crucial cell involved directly or indirectly in the induction of most immunologic functions (*3, 11*). Therefore, a functional defect of T4 cells would result in impairment of inductive signals to multiple components of the immune response and cause global immune defects.

HIV-1 selectively infects cells expressing the CD4 molecule (*1, 2*), resulting in substantial quantitative and qualitative defects in $CD4^+$ T lymphocyte function in patients with AIDS. Our study demonstrates that the $CD4^+$ T cell is the predominant cell harboring HIV-1 in the peripheral blood of infected individuals. These cells were actively expressing HIV-1 as determined by in situ hybridization to detect viral RNA, immunofluorescence to detect viral antigens, and limiting dilution analysis of cocultures. We have shown that the $CD4^+$ T cell in AIDS patients maintains expression of its surface CD4 molecule despite actively expressing HIV-1. These methods have allowed us to determine that the precursor frequency of $CD4^+$ T cells that is actively expressing HIV-1 in patients with AIDS is approximately 1/1000 cells. This frequency is consistent with the previous studies that found only 1/100,000 to 1/10,000 PBMCs expressing viral mRNA (*4*). In normal individuals, $CD4^+$ T cells make up 35 to 45% of Ficoll-Hypaque–separated PBMCs. In AIDS patients, $CD4^+$ T cells typically represent only 1 to 25% of total PBMCs. Thus, we have enriched the virus-containing $CD4^+$ T cells derived from PBMCs between 4- and 100-fold in AIDS patients. In this regard, one expects the frequency of in situ–positive $CD4^+$ T cells to be one to two logs higher than in PBMCs.

These methodologies however, are incapable of detecting cells that may be latently infected and not expressing viral RNA or protein. PCR, a technique that can determine the presence of cells containing either latent or actively replicating HIV-1, has both confirmed that the $CD4^+$ T cell is the reservoir for HIV-1 in the peripheral blood of infected individuals and enabled us to determine that the frequency of $CD4^+$ T cells that contain HIV-1 DNA is at least 1/100 cells in patients with AIDS. This represents a viral burden that is at least 100-fold greater than previously reported for AIDS patients. In contrast to individuals with AIDS, asymptomatic HIV-1–seropositive individuals have a frequency of HIV-1 DNA in $CD4^+$ T cells that is much less, on the order of 1/10,000 cells (*12*).

There are several reasons why our observation of the $CD4^+$ T cell as the predominant cell harboring HIV-1 in the peripheral blood is noteworthy, and in some respects, unexpected. First, it is well described that in vitro infection of $CD4^+$ T cells with HIV results in downmodulation and loss of the CD4 molecule on the surface of these cells (*2*). The sole exception to this observation is the recent report that a strain of HIV-2 does not lead to loss of CD4 expression on the target T cells (*13*). In addition, we have found that the ability to recover HIV-1 in culture from patients with AIDS is actually enhanced in those with the fewest number of circulating T cells. In a group of 88 patients with AIDS, we have noted a strong inverse correlation between the number of $CD4^+$ T cells and the production of RT activity in coculture (Pearson correlation coefficient $r = -0.7$, $P < 0.05$) (*14*); our findings support this observation. It appears that patients who are late in the course of disease will have a larger viral burden than early asymptomatic seropositives. Despite a

10-fold decline in $CD4^+$ T cells that is typically seen in the course of AIDS, the frequency of infection in these cells is at least 100-fold greater than in early seropositives. It is this high level of infection that may be the primary cause for the relentless and often accelerated decline in T4 cell number and function in patients with AIDS.

Evidence is accumulating to support the theory that monocytes and macrophages, subsets of which express the CD4 molecule (*15*), are involved in the pathogenesis of HIV-1 infection. It has been shown that monocytes can be infected in vitro with HIV-1, and the virus can be isolated from or identified in monocytes obtained from the blood as well as from cells of monocyte lineage in the brain and alveolar macrophages in the lung (*16–18*). Using PCR to examine highly enriched sorted monocytes from HIV-1–infected individuals, we have found that only a minority of such individuals have demonstrable HIV-1 in their peripheral monocytes, whereas all have HIV-1 DNA in their $CD4^+$ T cells. One may theorize that unlike the $CD4^+$ T cells and tissue macrophages, the relatively immature monocytes found in the peripheral blood are less susceptible to infection by HIV-1.

Having determined that the $CD4^+$ T cell is the primary cell in the peripheral blood that contains HIV-1, it will be important to determine what portion of these cells contain latent HIV-1 exclusively and to try and identify those properties of HIV-1 that maintain it in a latent state. In this regard, PCR may be used to amplify specific regulatory regions of HIV-1 such as *nef* (*3′-orf*) and to determine their level of transcriptional activation in different stages of infection in order to study factors that may control latency.

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19. Fluorescently labeled PBMCs were sorted with an EPICS C flow cytometer (Coulter Electronics) equipped with a 2-W argon laser with an excitation wavelength of 488 nm. Sorting logic was controlled by the use of forward angle light scatter versus log green fluorescence bit map gating. Cellular debris, red blood cells, and cell aggregates were eliminated from sorting by forward angle light scatter. The cells reactive with each monoclonal antibody were determined by comparing the fluorescence of cells stained with specific antibody and cells that had been incubated with FITC-labeled isotype controls. Cells were sorted at a rate of approximately 2000 cells per second with coincidence abort engaged. Both positively and negatively stained cells meeting the appropriate sort criterion were collected into separate tubes containing sterile phosphate-buffered saline (PBS). On completion of cell sorting, both positive and negative cell populations were analyzed for purity with machine parameters identical to those for sorting.
20. The RT assay used for these experiments was carried out as previously described [R. L. Willey *et al.*, *J. Virol.* **62**, 139 (1988)]. Briefly, 10 μl of culture supernatant was added to a cocktail containing polyadenylated oligo(dT) (Pharmacia), $MgCl_2$, and ^{32}P-labeled thymidine triphosphate (Amersham) and was incubated at 37°C for 2 hours. Five microliters of the cocktail was then spotted onto DE81 paper, dried, and washed in 1× saline sodium citrate buffer. The paper was dried and exposed to radiographic film for 12 hours at −80°C.
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23. Cells to be examined for HIV-1 DNA were washed and pelleted in PBS, and lysed in 0.001% Triton X-100/0.0001% SDS in TE buffer (10 m*M* tris-HCl, *p*H 8, and 0.5 m*M* EDTA, *p*H 8) with 600 μg per milliliter of proteinase K (Boehringer Mannheim) for 1 hour at 56°C, 15 min at 95°C. The PCR reaction mixture contained 50 μl of DNA lysate, 50 pmol each of primers, 200 μ*M* each of four deoxynucleotide triphosphates (Boehringer Mannheim), 10 m*M* tris-HCl (*p*H 8.3), 2.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.2% gelatin, and 2 units of thermostable DNA polymerase from *Thermus aquaticus* (Perkin-Elmer Cetus). Primer pairs used in these experiments included SK 68/69 (*env* 7801–7820, *env* 7922–7942), SK38/39 (*gag* 1551–1578, *gag* 1638–1665), SK29/30 (LTR 501–518, LTR 589–605), and QH26/27 (HLA-DQa control) as previously described (*6*). Amplification was carried out by means of a programmable thermal cycler (Perkin-Elmer, Cetus) with denaturation at 94°C, annealing at 55°C, and extension at 72°C for 30 cycles. After amplification, 20-μl aliquots were taken from HLA-DQa control tubes for agarose gel electrophoresis (3% NuSieve, 1% agarose) to determine if amplification had occurred. Aliquots of 30 μl were taken from the test amplification tubes, mixed with a ^{32}P-labeled adenosine triphosphate (ATP) end-labeled probe (SK70, *env* 7841 to 7875; SK19, *gag* 1595 to 1635; and SK31, LTR 552 to 585 probes for the *env*, *gag*, and LTR primers, respectively) (*6*), denatured at 94°C, and hybridized at 56°C for 15 min. The hybridized heteroduplexes were then analyzed on a 10% polyacrylamide gel. Autoradiograms of the gels were obtained by exposure of Kodak XAR film at −70°C at 2, 4, and 16 hours in the presence of an intensifying screen.

24. Funded in part with federal funds from the Department of Health and Human Services under contract number NO1-CO-74102. We thank M. A. Rust for editorial assistance.

9 January 1989; accepted 2 May 1989

Antibody to Interleukin-5 Inhibits Helminth-Induced Eosinophilia in Mice

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When rodents are infected with the nematode *Nippostrongylus brasiliensis*, large numbers of eosinophils appear in their blood and lungs and their serum immunoglobulin E (IgE) is increased. Injection of a monoclonal antibody to interleukin-5 completely suppressed the blood eosinophilia and the infiltration of eosinophils in the lungs of parasitized mice but had no effect on serum IgE. In contrast, an antibody to interleukin-4 inhibited parasite-induced IgE but not the eosinophilia. These results show that interleukin-5 is important in eosinophil production in vivo and that IgE and eosinophil production are regulated by different cytokines produced by the T_H2 subset of CD4-expressing T cells.

BLOOD AND TISSUE EOSINOPHILIA, large immunoglobulin E (IgE) responses, and intestinal mastocytosis are characteristic of the mammalian host response to helminth parasites (*1*). All three responses appear to be regulated by T lymphocytes since they do not occur in athymic (*2*) or T cell–depleted animals (*3*). Both mouse and human interleukin–5 (IL-5) have been shown to be potent and specific

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