ability of fixed cells to present peptides to CTLs and complements the extensive findings regarding the association of antigenic peptides with class II molecules (*14*).

By contrast, the inhibitory effect of brefeldin A on the presentation of viral proteins suggests that antigens processed by the cytosolic route associate with newly synthesized class I molecules prior to their exit from the trans-Golgi complex. To establish this point more firmly, it will be necessary to examine whether brefeldin A inhibits other processes potentially involved in antigen presentation, such as proteolysis, translocation of antigen into an exocytic compartment, and exocytosis of antigen not mediated by class I molecules. The idea that processed antigens associate with newly synthesized class I molecules is attractive, however, as it is consistent with (i) the parallel effects of brefeldin A on class I molecule transport and antigen presentation in the temperature reversal experiments, (ii) the inhibition of antigen presentation mediated by adenovirus E3/19K glycoprotein, which specifically interacts with newly synthesized class I molecules and blocks their intracellular transport (*15*), and (iii) our recent findings that prolonged incubation of cells with protein synthesis inhibitors (4 to 8 hours) prior to their sensitization with noninfectious influenza virus inhibits the K^k-associated presentation of HA and NP [but not NP(50–63) peptide] without affecting the amount of K^k present on the cell surface (*16*).

REFERENCES AND NOTES

1. A. R. M. Townsend *et al.*, *Cell* **44**, 959 (1986).
2. J. W. Yewdell, J. R. Bennink, Y. Hosaka, *Science* **239**, 637 (1988).
3. M. W. Moore, F. R. Carbone, M. J. Bevan, *Cell* **54**, 777 (1988).
4. R. M. Zinkernagel and P. C. Doherty, *Adv. Immunol.* **27**, 51 (1979).
5. A. Takatsuki and G. Tamura, *Agric. Biol. Chem.* **49**, 899 (1985); Y. Misumi *et al.*, *J. Biol. Chem.* **261**, 11398 (1986); J. A. Magner and E. Papagiannes, *Endocrinology* **122**, 912 (1988).
6. T. Fujiwara, K. Oda, S. Yokota, A. Takatsuki, Y. Ikehara, *J. Biol. Chem.* **263**, 18545 (1988); J. Lippincott-Schwartz, L. C. Yuan, J. S. Bonifacino, R. D. Klausner, *Cell* **56**, 801 (1989); R. W. Doms, G. Russ, J. W. Yewdell, *J. Cell Biol.*, in press.
7. J. R. Bennink, J. W. Yewdell, G. L. Smith, B. Moss, *J. Virol.* **61**, 1098 (1987); J. R. Bennink and J. W. Yewdell, *J. Exp. Med.* **168**, 1935 (1988).
8. Y. Hosaka, F. Sasao, K. Yamanaka, J. R. Bennink, J. W. Yewdell, *J. Immunol.* **140**, 606 (1988).
9. J. Bastin, J. Rothbard, J. Davey, I. Jones, A. Townsend, *J. Exp. Med.* **165**, 1508 (1987).
10. In additional experiments, we found that treatment of cells with brefeldin A for as long as 5 hours before sensitization with peptide did not alter their recognition by NP-specific CTLs.
11. M.-J. Gething, K. McCammon, J. Sambrook, *Cell* **46**, 939 (1986); J. W. Yewdell, A. Yellen, T. Bachi, *ibid.* **52**, 843 (1988); C. S. Copeland *et al.*, *ibid.* **53**, 197 (1988).
12. G. Russ, J. Bennink, J. W. Yewdell, unpublished results.
13. D. B. Williams, S. J. Swiedler, G. W. Hart, *J. Cell Biol.* **101**, 725 (1985).
14. R. H. Schwartz, *Adv. Immunol.* **38**, 31 (1986); E. R. Unanue and P. M. Allen, *Science* **236**, 551 (1987); J. A. Berzofsky *et al.*, *Immunol. Rev.* **98**, 9 (1988).
15. H.-G. Burgert and S. Kvist, *Cell* **41**, 987 (1985); M. Andersson, S. Paabo, T. Nilsson, P. A. Peterson, *ibid.* **43**, 215 (1985); Y. Tanaka and S. S. Tevethia, *Virology* **165**, 357 (1988).
16. J. W. Yewdell and J. R. Bennink, unpublished results.
17. Brefeldin A is not commercially available. Natural brefeldin A was provided by A. Takatsuki (University of Tokyo) and C. R. Hutchinson (University of Wisconsin); synthetic brefeldin A was provided by E. J. Corey (Harvard University). Brefeldin A was diluted from a stock solution of 10 mg/ml in methanol that was stored at −20°C. The concentration of brefeldin A used in experiments was adjusted to account for the twofold difference in potency observed between the different batches. In preliminary experiments, we found that more brefeldin A was required to initiate the blockade of antigen presentation than to maintain it. Reducing the concentration of brefeldin A in the ^{51}Cr release assay, which requires relatively large quantities of media, allowed us to conserve our limited supply of brefeldin A.
18. K. B. Eager *et al.*, in preparation.
19. B. E. Coupar, M. E. Andrew, D. B. Boyle, R. V. Blanden, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7879 (1986).
20. L. C. Eisenlohr, W. Gerhard, C. J. Hackett, *J. Virol.* **61**, 1375 (1987).

21. We thank A. Takatsuki, C. R. Hutchinson, and E. J. Corey for brefeldin A; D. Boyle (CSIRO, Geelong; Victoria, Australia) for the K^d-Vac recombinant; G. Russ for critical reading of this manuscript; and M. Mankowski for excellent technical assistance.

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Involvement of a Leukocyte Adhesion Receptor (LFA-1) in HIV-Induced Syncytium Formation

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Cell fusion (syncytium formation) is a major cytopathic effect of infection by human immunodeficiency virus (HIV) and may also represent an important mechanism of $CD4^+$ T-cell depletion in individuals infected with HIV. Syncytium formation requires the interaction of CD4 on the surface of uninfected cells with HIV envelope glycoprotein gp120 expressed on HIV-infected cells. However, several observations suggest that molecules other than CD4 play a role in HIV-induced cell fusion. The leukocyte adhesion receptor LFA-1 is involved in a broad range of leukocyte interactions mediated by diverse receptor-ligand systems including CD4–class II major histocompatibility complex (MHC) molecules. Possible mimicry of class II MHC molecules by gp120 in its interaction with CD4 prompted an examination of the role of LFA-1 in HIV-induced cell fusion. A monoclonal antibody against LFA-1 completely inhibited HIV-induced syncytium formation. The antibody did not block binding of gp120 to CD4. This demonstrates that a molecule other than CD4 is also involved in cell fusion mediated by HIV.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS), a fatal disease characterized by profound immunosuppression, opportunistic infections, and neuropathies (*1*). Although only a small fraction of circulating lymphocytes are infected with the virus (*2*), there is a marked loss of T cells bearing the virus receptor CD4 (*3, 4*). The depletion of $CD4^+$ T cells appears to contribute significantly to the immunosuppression associated with AIDS. Syncytium formation resulting from HIV-induced cell fusion has been shown to be the primary cytopathic effect of the virus in vitro (*5*) and has been postulated to account for the loss of $CD4^+$ T cells in vivo (*6–8*).

CD4, through its interaction with the HIV envelope glycoprotein gp120, plays an important role in syncytium formation (*6, 9–12*). However, several observations suggest that molecules on the surface of uninfected cells other than CD4 are also involved in HIV-induced cell fusion. First, fusion of HIV-infected cells to uninfected cells does not correlate with CD4 density on the surface of the uninfected cells (*13, 14*). In addition, whereas transfection of nonlymphoid human cells with CD4 renders such cells capable of fusion to HIV-infected cells, this is not true for CD4-transfected mouse cells (*15*). Finally, there is a disparity in the capacity of sera from AIDS patients to block binding of HIV particles to $CD4^+$ cells and the capacity of the same sera to block fusion of HIV-infected cells to $CD4^+$ uninfected cells (*11*).

CD4 interacts directly with class II major histocompatibility complex (MHC) molecules in class II MHC–restricted T helper cell responses (*16, 17*). The involvement of the leukocyte adhesion receptor (LAR) LFA-1 in such responses has been demonstrated with monoclonal antibodies (MAbs)

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to LFA-1 (anti–LFA-1) (*18–20*). Structural similarities between gp120 and class II MHC molecules (*21*) suggested to us that the binding of gp120 to CD4 may mimic the interaction between class II MHC molecules and CD4 (*16, 17*). We therefore examined the role of LAR in HIV-mediated cell fusion.

For this purpose, we developed a syncytium formation assay that involved phytohemagglutinin (PHA)-stimulated T cells (PHA-blasts) and a cell line designated 8E5. This CD4− cell line was selected from surviving clones after HIV infection of the A3.01 cell line, a subclone of the CEM T cell line (*22, 23*). The 8E5 cells carry a single copy of the entire LAV genome but produced noninfectious virus particles because of a point mutation in the reverse transcriptase gene (*24*). The 8E5 cells express HIV envelope glycoproteins (*23*) and, when mixed with CD4-positive PHA-blasts and T cell lines, produce cytopathic effects identical to those observed in cultures of T cells infected with wild-type virus (*23*). In our assay, syncytia or balloon cells consisting of 10 to 50 or more fused cells from between 4 and 10 hours after mixing 8E5 cells with PHA-blasts and $CD4^+$ T cell lines, respectively (*25*). Continued incubation results in rapid syncytia death as determined by vital dye exclusion.

To determine their effect on HIV-mediated cell fusion, MAbs against human leukocyte antigens were added to cocultures of PHA-blasts and 8E5 cells. H52, a MAb against the β-subunit of the LFA-1 (CD18) (*18*), completely inhibited syncytium formation (Fig. 1). Fusion was also blocked by a MAb (MHM.24) against the α-subunit of LFA-1 (CD11a) (*18, 20*) (Fig. 1). However, the MAb MHM.24 was less effective than MAb H52, since very small syncytia were rarely observed. H5A4, a MAb against a different member of the LAR family, Mac-1 (complement receptor type 3; CD11b) (*18*), had no effect on the fusion of 8E5 cells to the PHA-blasts. Also, fusion was not affected by two MAbs recognizing unrelated cell surface proteins: MHM.5, anti-class I MHC (*26*), and H5A5, anti-leukocyte common antigen (CD45) (Fig. 1). Because these two antigens are expressed at equal or higher densities than LAR on PHA-blasts (*25*), the failure of the latter two antibodies to block fusion suggests that inhibition by antibodies to LAR was not due to a nonspecific steric effect.

Leu3a, a MAb against CD4, which has been previously shown to block binding of gp120 to CD4 (*9*), completely inhibited fusion of 8E5 to PHA-blasts (Fig. 1). Inhibition of fusion by Leu3a and the absence of fusion between the PHA-blasts and uninfected A3.01 cells (Fig. 1, control) confirmed that the fusion was mediated by HIV. A number of commercially available MAbs against gp120 failed to inhibit fusion in our assay system, consistent with results obtained by other investigators using a different assay for syncytium formation (*27*).

PHA-blasts and the 8E5 cells formed very large aggregates within 1 hour of mixing in the syncytium assay. Formation of these aggregates was completely inhibited by H52, MHM.24, and Leu3a but not by the other MAbs (*25*). This observation suggests that LFA-1 like CD4 is involved in an early adhesion step in syncytium formation.

The inhibition of syncytium formation by MAb H52 was observed whether the PHA-blasts were mixed with 8E5 cells infected with the mutant virus or the CEM T cell line infected with wild-type HIV ($HTLV\text{-}III_B$) (*25*). This result showed that the effect of anti–LFA-1 on syncytium formation was not restricted to cells infected with the mutant virus.

The H52 MAb blocked 8E5–PHA-blast fusion in a dose-dependent manner, with complete inhibition observed at concentrations above 3 μg/ml (Fig. 2). The inhibition of LFA-1–mediated lymphocyte adhesion functions by MAb H52 shows a very similar dose dependency (*18, 28*). PLM-2, a MAb against a CD18 epitope not associated with LFA-1 adhesion functions (*28*), did not significantly affect fusion at any concentration (Fig. 2).

We have previously shown that inhibition of lymphocyte interactions by anti–LFA-1 is a unidirectional effect even when both cell types express LFA-1 (*20*). To determine whether the effect of MAb to LFA-1 on syncytium formation was also unidirectional, we first analyzed LFA-1 expression by flow cytometry. Both 8E5 cells and PHA-blasts expressed LFA-1, although the

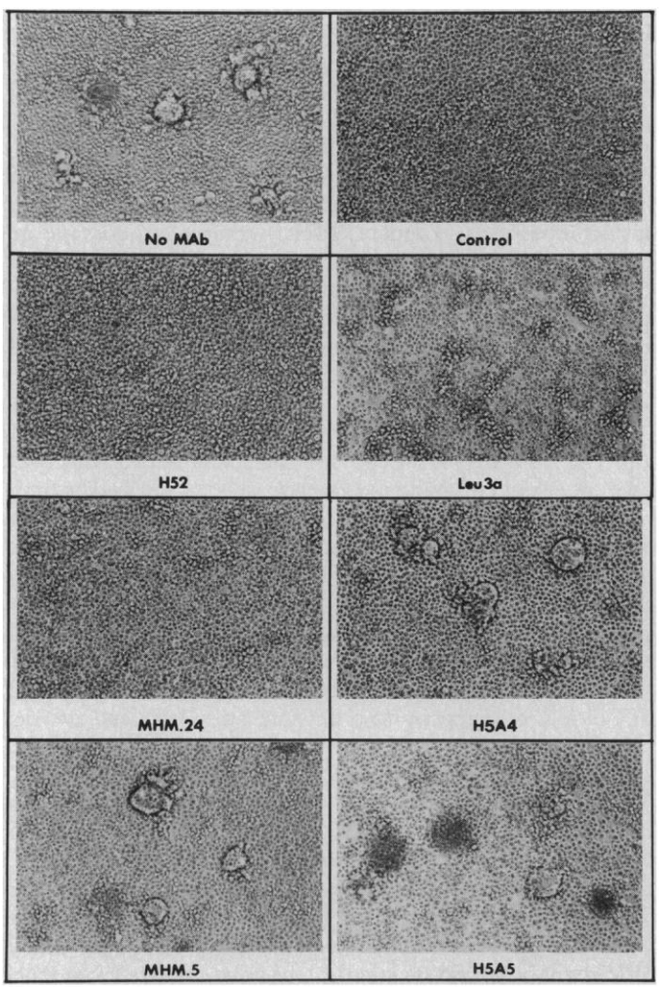

Fig. 1. Inhibition of syncytium formation by MAbs. The effect of MAbs on the fusion of 8E5 cells to PHA-blasts was determined in a syncytium formation assay. The MAbs tested were: H52, anti-CD18 (LFA-1β); MHM.24, anti-CD11a (LFA-1α); H5A4, anti-CD11b (Mac-1 α); H5A5, anti-CD45 (leukocyte common antigen); MHM.5, anti–HLA-A, B, C; and Leu3a, anti-CD4. All antibodies are immunoglobulin G1k (IgG1k) isotype. The 8E5 and A3.01 cell lines were maintained in complete medium (RPMI 1640 supplemented with 10% fetal bovine serum (HyClone) and 10 m*M* Hepes). The 8E5 cell line, a surviving clone of infected A3.01 cells, expresses HIV glycoproteins but produces noninfectious virus because of a mutant reverse transcriptase gene (*22–24*). PHA-blasts were generated by incubating peripheral blood mononuclear cells for 3 days in the presence of PHA (Wellcome Diagnostics) at a concentration of 0.25 μg/ml in complete medium. Cells were washed three times with phosphate-buffered saline (PBS) and resuspended in complete medium at a density of 5×10^6 per milliliter. MAbs were used in the form of purified IgG at a concentration of 25 μg/ml. PHA-blasts were mixed with an equal volume (30 μl) of MAb or medium in the wells of half-area 96-well plates (Costar) and incubated for 30 min at 25°C. Thirty microliters of 8E5 cells were then added, followed by incubation for 10 hours at 37°C in a humidified CO_2 incubator. Control wells consisted of PHA-blasts incubated with an equal number of uninfected A3.01 cells.

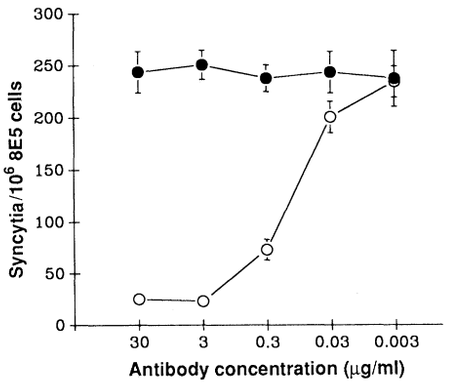

Fig. 2. Dose-dependent inhibition of syncytium formation by H52 IgG. PHA-blasts, generated as described in Fig. 1, were incubated with various concentrations of purified H52 or PLM-2 IgG before the addition of 8E5 cells. PLM-2 is an IgG1k MAb against CD18 that does not inhibit LFA-1–mediated functions (*28*). The assay was carried out exactly as described in Fig. 1. Syncytia were counted on an inverted microscope with a low power objective (40×) after adding trypan blue (0.1%). Data shown are the mean syncytia count/10^6 8E5 cells of duplicate wells. Similar results were obtained in each of three experiments.

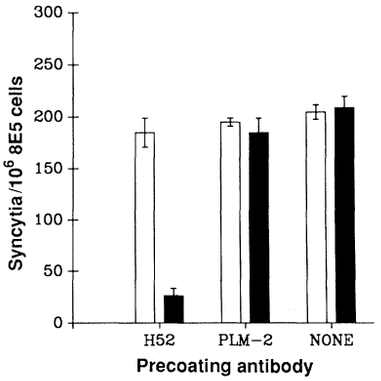

Fig. 3. H52 blocks syncytia formation at the level of PHA-blasts, not 8E5 cells. PHA-blasts and 8E5 cells (2.5×10^6) were incubated for 1 hour on ice in 0.5 ml of complete medium alone or complete medium containing purified H52 or PLM-2 IgG at 25 μg/ml. After pelleting the cells, unbound MAb was removed by washing two times with 10 ml of PBS. The antibody-coated PHA blasts and 8E5 cells were then resuspended in complete medium and mixed with uncoated 8E5 cells and PHA blasts, respectively, followed by incubation at 37°C for 10 hours as described in Fig. 1. Syncytia formation was scored as described in Fig. 2. Data shown are mean syncytia/10^6 8E5 cells (± SEM) of duplicate wells. The experiment was carried out three times with similar results.

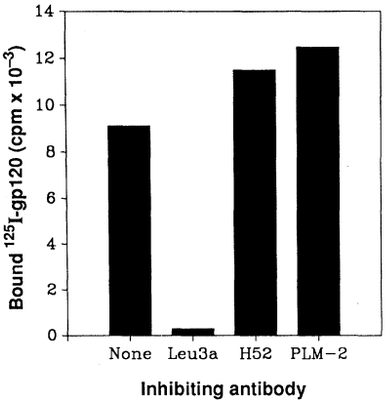

Fig. 4. Inhibition of gp120 binding to CEM cells by MAb. gp120 was purified as follows. HIV (*35*) was pelleted (110,000*g*, 1.5 hours) from culture supernatants of infected PHA-blast cells and washed once with PBS. The virus was resuspended in PBS and spun in a vortex mixer vigorously to shear off gp120 followed by centrifugation at 110,000*g*. The resulting supernatant was concentrated with a 30,000-dalton cut-off Centricon filter. The retained proteins, which consisted primarily of gp120 and bovine serum albumin (BSA, 10 to 30%), were radioiodinated by the standard chloramine-T method. The labeled proteins (2 to 5 μCi/μg) were diluted in PBS containing a high concentration of BSA (2%) to eliminate binding of ^{125}I-labeled BSA. $CD4^+$ CEM cells (5×10^5) were preincubated with Leu3A, H52, and PLM-2 MAb at 25 μg/ml in complete medium (see Fig. 1) before adding 50 ng of radioiodinated gp120. After a 1-hour incubation at 0°C, the cells were washed twice, and bound radiolabel was measured. Background binding was determined by preincubating cells with a 200-fold excess of unlabeled gp120. Data shown are mean bound counts per minute (SEM <15%) above background of triplicate assay tubes. Similar results were obtained in each of two experiments.

expression on 8E5 was substantially less than on the blasts (*25*). Each cell type was coated with MAb H52 or the control MAb PLM-2 and washed to remove unbound MAb before the assay of syncytium formation. Coating PHA-blasts with H52 resulted in near complete inhibition of fusion, whereas similar treatment of the 8E5 cells had essentially no effect (Fig. 3). Fusion was not affected by coating either the PHA-blasts or the 8E5 cells with the control MAb. This result showed that anti–LFA-1 blocked fusion at the level of the PHA-blast, not the HIV-infected 8E5 cells, suggesting that LAR on the $CD4^+$ cells interacted with a ligand expressed on 8E5 cells.

Nonspecific agents such as dextran sulfate that block the interaction of gp120 with CD4 by steric effects are known to inhibit HIV-mediated cell-cell fusion (*29*). We therefore tested whether the binding of MAb H52 to LFA-1 on the surface of $CD4^+$ cells interfered with the binding of gp120 to CD4. Consistent with previous findings, cells coated with the Leu3a MAb (anti-CD4) showed essentially zero binding of gp120 (*9*) (Fig. 4). In contrast, coating cells with either mAB H52 or the control MAb PLM-2 had no inhibitory effect on the binding of gp120. This result demonstrated that inhibition of syncytium formation by MAb H52 was not due to interference with HIV receptor function, since binding of gp120 to CD4 was not blocked by this MAb.

Although our data indicate that LFA-1 is involved in HIV-mediated cell fusion, elucidation of the exact role of LFA-1 in this process will require further study. The results presented herein support the notion that gp120 may mimic class II MHC molecules in its interaction with CD4. However, structural homology between gp120 and class II MHC need not form the basis of the interaction between gp120 and CD4 or the observed inhibition of syncytium formation by anti–LFA-1. The participation of LFA-1 in a broad range of cell-cell interactions mediated by a variety of cell surface receptors has led to the postulate that LFA-1 may serve as an accessory adhesion receptor that stabilizes weak interactions (initiated by other receptors) between cells (*19*). The observed inhibition of adhesion between 8E5 cells and PHA-blasts by MAb H52 in the present study suggests that LFA-1 may play a similar role in the interaction between HIV-infected cells and $CD4^+$ cells. There is strong evidence indicating that HIV envelope glycoprotein gp41 serves as the fusion protein for HIV in a manner similar to fusion proteins of other viruses (*30, 31*), suggesting that one or more cell surface molecules other than CD4 may serve as fusion receptors on uninfected cells. Although we have demonstrated in the present study that MAb H52 does not inhibit the binding of gp120 to CD4, our data do not rule out blockage of the putative HIV fusion receptor or receptors by this MAb. Our recent observation that MAb H52 inhibits the fusion of macrophages and formation of multinucleated giant cells (*32*) supports this contention. However, preliminary studies with a MAb against ICAM-1, an LFA-1 ligand (*19*), suggest that LFA-1 on PHA-blasts interacts with ICAM-1 on 8E5 cells (*25*).

Whereas our data suggest that anti–LFA-1 may block direct cell to cell transmission of HIV, the highly immunosuppressive nature of antibodies to LFA-1 would probably limit their usefulness as therapeutic agents against AIDS. However, if bone marrow transplantation is pursued as a therapeutic modality in the treatment of the disease, these MAbs may be very useful in preventing the spread of virus in the immunosuppressed host by inhibiting fusion of residual infected cells to the transplanted cells. In addition, since higher infectivity and replication of HIV is associated with activation of T cells (*33*) and macrophages (*34*), the immunosuppressive properties of anti–LFA-1 may represent a second beneficial effect in this setting. The results of the present study provide direct evidence that cell surface molecules other than CD4 are

involved in HIV-induced cell fusion, an important mechanism in the pathogenesis of AIDS, and may pave the way for broader therapeutic strategies than those currently in development.

REFERENCES AND NOTES

1. A. S. Fauci, *Science* **239**, 617 (1988).
2. M. E. Harper, L. M. Marselle, R. C. Gallo, F. Wong-Staal, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 772 (1986).
3. J. S. McDougal *et al.*, *J. Immunol.* **135**, 3151 (1985).
4. D. Klatzmann *et al.*, *Science* **225**, 59 (1984).
5. W. A. Haseltine and J. G. Sodroski, in *Acquired Immunodeficiency Syndrome*, J. C. Gluckman and E. Vilmer, Eds. (Elsevier, New York, 1987), pp. 47–56.
6. J. D. Lifson, G. R. Reyes, M. S. McGrath, B. S. Stein, E. G. Engleman, *Science* **232**, 1123 (1986).
7. D. C. Montefiori and W. M. Mitchell, *Virology* **160**, 372 (1987).
8. B. Yoffe *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1429 (1987).
9. A. G. Dalgleish *et al.*, *Nature* **312**, 763 (1984).
10. J. S. McDougal *et al.*, *Science* **231**, 382 (1986).
11. J. D. Lifson *et al.*, *Nature* 323, 725 (1986).
12. J. Sodroski, W. C. Goh, C. Rosen, K. Campell, W. A. Haseltine, *ibid.* **322**, 470 (1986).
13. M. Somasundaran and H. L. Robinson, *J. Virol.* **61**, 3114 (1987).
14. R. Kikukawa *et al.*, *ibid.* **57**, 1159 (1986).
15. P. J. Maddon *et al.*, *Cell* **47**, 333 (1986).
16. C. Doyle and J. L. Strominger, *Nature* **330**, 256 (1987).
17. D. Gay *et al.*, *ibid.* **328**, 626 (1987).
18. J. E. K. Hildreth and J. T. August, *J. Immunol.* **134**, 3272 (1985).
19. T. A. Springer, M. L. Dustin, T. K. Kishimoto, S. D. Marlin, *Annu. Rev. Immunol.* **5**, 223 (1987).
20. J. E. K. Hildreth, F. M. Gotch, P. D. K. Hildreth, A. J. McMichael, *Eur. J. Immunol.* **13**, 202 (1983).
21. H. Golding *et al.*, *J. Exp. Med.* **167**, 914 (1988).
22. T. M. Folks *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4539 (1985).
23. T. M. Folks *et al.*, *J. Exp. Med.* **164**, 280 (1986).
24. H. E. Gendelman *et al.*, *Virology* **160**, 323 (1987).
25. J. E. K. Hildreth, unpublished observations.
26. S. A. Ellis, C. Taylor, J. E. K. Hildreth, A. J. McMichael, *Human Immunol.* **13**, 13 (1985).
27. L. A. Lasky *et al.*, *Cell* **50**, 975 (1987).
28. J. E. K. Hildreth, V. Holt, J. T. August, M. D. Pescovitz, *Mol. Immunol.*, in press.
29. H. Mitsuya *et al.*, *Science* **240**, 646 (1988).
30. M. Kowalski *et al.*, *ibid.* **237**, 1351 (1987).
31. W. R. Gallaher, *Cell* **50**, 327 (1987).
32. J. E. K. Hildreth and R. J. Orentas, in preparation.
33. J. B. Margolick, D. J. Volkman, T. M. Folks, A. S. Fauci, *J. Immunol.* **138**, 1719 (1987).
34. Y. Koyanagi *et al.*, *Science* **241**, 1673 (1988).

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Sporozoite Vaccine Induces Genetically Restricted T Cell Elimination of Malaria from Hepatocytes

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The target of the CD8$^+$ T cell–dependent immunity that protects mice immunized with irradiation-attenuated malaria sporozoites has not been established. Immune BALB/c mice were shown to develop malaria-specific, CD8$^+$ T cell–dependent inflammatory infiltrates in their livers after challenge with *Plasmodium berghei* sporozoites. Spleen cells from immune BALB/c and C57BL/6 mice eliminated hepatocytes infected with the liver stage of *P. berghei* in vitro. The activity against infected hepatocytes is not inhibited by antibodies to interferon-γ and is not present in culture supernatants. It is genetically restricted, an indication that malaria antigens on the hepatocyte surface are recognized by immune T effector cells. Subunit vaccine development will require identification of the antigens recognized by these T cells and a method of immunization that induces such immunity.

AFTER INOCULATION, MALARIA SPOROZOITES are in the peripheral circulation for less than an hour before they enter the liver where, during several days, they develop to mature liver-stage parasites that rupture and release merozoites that invade erythrocytes. Immunization with irradiation-attenuated malaria sporozoites (irr-spz) protects animals (*1*) and humans (*2*) against challenge with normal sporozoites. In some strains of mice the effector arm of this protective immunity has been shown to require CD8$^+$ lymphocytes and interferon-γ (*3, 4*). Such immunity does not protect against challenge with erythrocyte-stage parasites (*1*) or liver-stage merozoites (*5*), and circulating sporozoites are an unlikely target for a protective CD8$^+$ T cell–dependent immune response that would require cell surface presentation of antigen in combination with class I major histocompatibility (MHC) proteins. The infected hepatocyte is the likely target for such immunity. However, most workers still refer to sporozoite vaccine development, since there is little or no inflammatory reaction around most mature parasites in the livers of naïve animals after infection (*6, 7*) (Fig. 1A), and the presence of malaria antigens on the surface of hepatocytes has not been demonstrated. In the current studies we show that multiple, malaria-specific inflammatory reactions occur in the livers of immune mice and that T cells from immune mice eliminate liver-stage parasites from hepatocyte cultures.

We immunized BALB/c mice with a single dose of *Plasmodium berghei* irr-spz. Two weeks later the mice were infected with sporozoites, and 43 hours later they were killed. Multiple inflammatory infiltrates (Fig. 1B) were found in the livers of mice immunized with irr-spz and challenged with normal sporozoites, but few infiltrates were found in controls (*8*). In addition, the response was specific for malaria (Table 1). The infiltrates contained numerous macrophages, polymorphonuclear leukocytes, eosinophils, and CD8$^+$ lymphocytes (Fig. 1C). CD4$^+$ lymphocytes were present in lower concentration, and the number of Kupffer cells recognized by monoclonal antibody SER-4 was similar in infiltrates and normal hepatic tissue (*8*). To further define the infiltrates, we adoptively transferred spleen cells from immune or naïve donors into naïve recipients, challenged the recipients, and examined their livers 43 hours later (*9*). Infiltrates were present in the livers of mice that received unfractionated immune spleen cells (ISCs) (5.2 ± 4.19 infiltrates per 20 low-power fields, mean ± SD) and CD4$^-$ ISCs (5.5 ± 2.12), but not in those that received CD8$^-$ ISCs (0) or normal spleen cells (0.3 ± 0.57). The numbers of infiltrates in these experiments exceeded the numbers of mature schizonts expected after such a sporozoite challenge.

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