resolution) of HIV RT complexed with duplex DNA (*24*). Within the intrinsic limitations of the experiments, our model is not inconsistent with most of the results describing DNA binding to KF (*5, 7, 8, 25*).

The angle of the DNA helix axis in cleft 2 with respect to the polymerase cleft (cleft 1) is unexpected. If one constructs a model that preserves most of the observed contacts between the duplex DNA and the protein, the model-built DNA requires a bend of about 80° to enter the polymerase cleft (Fig. 5). Such protein-induced bending of duplex DNA is not unknown. The crystal structure of a catabolite gene activator protein–DNA complex shows that the DNA is bent by 90°, which is achieved primarily through two sharp, 43° kinks (*26*). Several recent electron microscopy studies suggest that DNA is greatly bent (as much as 180°) when bound to RNA polymerase (*27*) as well as to UvrB (*28*).

We anticipate that the growing nascent strand of the DNA substrate can shuttle between the polymerase and the exonuclease active sites (Fig. 5), as has been previously proposed (*2–4, 29*) and for which there is some evidence (*6*). The destabilization of duplex DNA that would result from the anticipated DNA bending at the polymerase active site would make the equilibrium between single- and double-stranded DNA at the primer terminus more sensitive to mismatched base pairs. It will be interesting to see whether those DNA polymerases that do not contain an editing function bind straight duplex DNA, as does HIV RT (*23, 24*), whereas those with an editing exonuclease bind bent DNA, as appears likely with DNA Pol I.

REFERENCES AND NOTES

1. D. L. Ollis *et al.*, *Nature* **313**, 762 (1985); D. L. Ollis *et al.*, *ibid.*, p. 818.
2. C. M. Joyce and T. A. Steitz, *Trends Biochem. Sci.* **12**, 288 (1987).
3. T. A. Steitz *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **52**, 465 (1987).
4. P. S. Freemont *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8924 (1988).
5. M. Cowart *et al.*, *Biochemistry* **28**, 1975 (1989).
6. C. M. Joyce, *J. Biol. Chem.* **264**, 10858 (1989).
7. C. E. Catalano, D. J. Allen, S. J. Benkovic, *Biochemistry* **29**, 3612 (1990).
8. C. R. Guest *et al.*, *ibid.* **30**, 8759 (1991).
9. M. Delarue *et al.*, *Protein Eng.* **3**, 461 (1990).
10. L. S. Beese, J. M. Friedman, T. A. Steitz, in preparation.
11. A. H. Polesky *et al.*, *J. Biol. Chem.* **265**, 14579 (1990); A. H. Polesky *et al.*, *ibid.* **267**, 8417 (1992).
12. C. M. Joyce, unpublished data.
13. J. Warwicker, D. Ollis, F. M. Richards, T. A. Steitz, *J. Mol. Biol.* **186**, 645 (1985).
14. The sequences of the DNA oligonucleotides co-crystallized with KF are

    5′-TGCCTCGCGGCC-3′

    3′- GCGCCGG-5′

    Complementary 7-nt and 12-nt strands of DNA were annealed and incubated with a fivefold excess of 2′,3′ epoxyATP in a 3:1 molar ratio of DNA to protein under previously described conditions (*7*). The protein concentration in the reaction mixture was 10 mg/ml. The reaction mixture was incubated for 2 days at 10°C. The complex was crystallized at 17°C by vapor diffusion of a solution (10 to 12 mg/ml) of KF containing from 35 to 40% (w/v) saturated ammonium sulfate, 200 mM citrate buffer (pH 6.8), 20 mM $MgSO_4$, and 1 mM $ZnSO_4$ against a solution that was double in the concentration of all compounds except the KF:DNA complex. Crystals appeared after 6 to 8 weeks. A mutant protein [Asp^{355} → Ala (D355A)] with reduced 3′ to 5′ exonuclease activity (*30*) was used to reduce the possibility that the exonuclease would degrade the DNA in the cocrystals. The observed structure is isomorphous with the wild-type enzyme except at the exonuclease active site, where one of the two metal ions essential for exonuclease activity fails to bind. The remaining metal ion is the one that was not observed previously in a substrate complex because the D424A mutant was used to prevent substrate hydrolysis. Its position relative to the substrate is as had been expected from the positions of the two metal ions in the nucleoside monophosphate product complex (*15, 30*).
15. L. S. Beese and T. A. Steitz, *EMBO J.* **10**, 25 (1991).
16. An alternative interpretation of the two 7-nt molecules complexed to a 12-nt molecule results in a GT base pair at position 7 and a CC mismatch at position 5, where the electron density shows a good base pair. DNA from crystals used in the x-ray data collection were labeled with ^{32}P and analyzed by electrophoresis on denaturing gels. DNA lengths between 5 and 12 nt were observed, which suggests that some nuclease and polymerase processing of the DNA occurred.
17. J. M. Friedman, L. S. Beese, T. A. Steitz, unpublished data.
18. R. Marmorstein *et al.*, *Nature* **356**, 408 (1992).
19. L. Blanco *et al.*, *Gene* **100**, 27 (1991).
20. S. Basu *et al.*, *Biochemistry* **27**, 6710 (1988).
21. L. S. Beese and T. A. Steitz, in preparation.
22. T. I. Kolocheva *et al.*, *FEBS Lett.* **248**, 97 (1989).
23. L. A. Kohlstaedt, J. Wang, J. M. Friedman, P. A. Rice, T. A. Steitz, *Science* **256**, 1783 (1992).
24. E. Arnold *et al.*, *Nature* **357**, 85 (1992).
25. C. E. Catalano and S. J. Benkovic, *Biochemistry* **28**, 4374 (1989).
26. S. C. Schultz, G. C. Shields, T. A. Steitz, *Science* **253**, 1001 (1991).
27. B. ten Heggeler-Bordier, W. Wahli, M. Adrian, A. Stasiak, J. Dubochet, *EMBO J.* **11**, 667 (1992).
28. Q. Shi, R. Thresher, A. Sancar, J. Griffith, *J. Mol. Biol.* **226**, 425 (1992).
29. D. Brutlag and A. Kornberg, *J. Biol. Chem.* **247**, 241 (1972).
30. V. Derbyshire *et al.*, *EMBO J.* **10**, 17 (1991).
31. A. P. Xuong, S. T. Freer, R. Hamlin, D. Neilsen, W. Vernon, *Acta Crystallogr. A* **34**, 289 (1978).
32. A. T. Brunger, *X-PLOR Manual 2.0* (1990).
33. X-ray diffraction data were measured with a Xuong-Hamlin Mark II two-dimensional, position-sensitive area detector (*31*). The data are 87% complete to 3.2 Å, with a merging residual index (*R*) factor of 5.2%. The protein phases were obtained by rigid body refinement with the use of X-PLOR (*32*); phases were from the independently refined atomic model of the KF:DNA cocrystal in which DNA was disordered and not visible in the electron density maps although the conformational change in the thumb subdomain was observed (L. S. Beese and T. A. Steitz, unpublished data) followed by constrained positional refinement.
34. M. L. Connoly, *J. Mol. Graphics* **3**, 19 (1985).
35. There is a break in the electron density defining the main chain in this region, so it is difficult to be certain of the amino acid sequence. The electron density does not unambiguously determine which side chains are interacting with the DNA.

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Preferential Migration of Activated CD4$^+$ and CD8$^+$ T Cells in Response to MIP-1α and MIP-1β

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Recombinant human macrophage inflammatory protein–1α (rhMIP-1α) and rhMIP-1β were potent chemoattractants of human T lymphocytes. These rhMIP-1 cytokines attracted only T cells activated by monoclonal antibody to CD3 and did not attract unstimulated lymphocytes. Phenotypic analysis revealed that CD4$^+$ T cells were capable of migrating in response to rhMIP-1β, whereas rhMIP-1α induced chemotaxis of predominantly CD8$^+$ T lymphocytes. Activated naïve and memory T cells also migrated in response to rhMIP-1 cytokines. Furthermore, these cytokines enhanced the ability of T cells to bind to an endothelial cell monolayer. These results suggest that rhMIP-1 cytokines preferentially recruit specific T cell subsets during the evolution of the immune response.

The accumulation of leukocytes at sites of inflammation is induced by the local production and secretion of chemotactic ligands by a wide variety of stimulated cell types. Recently, several host-derived cytokines (chemokines) have been identified that stimulate chemotaxis in vitro and elicit the accumulation of various types of inflammatory cells in vivo (*1*). Neutrophils are preferentially induced to migrate by interleukin-8 [IL-8, also called neutrophil attracting peptide–1 (NAP-1)], melanoma growth stimulating activity (MGSA, also called GRO), NAP-II, and ENA-78, whereas monocytes are preferentially in-

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duced to migrate by macrophage chemotactic activating factor [MCAF, also called monocyte chemotactic protein–1 (MCP-1)], RANTES, I-309, MIP-1α, and MIP-1β (*1, 2*). Neutrophils and monocytes participate in acute and chronic nonimmunological inflammatory reactions; T lymphocytes, however, are crucial in delayed-type hypersensitivity responses. Purified RANTES (*2*), IL-8 (*3*), IL-2 (*4*), and IL-1 (*4*) have been reported to show in vitro T cell chemotactic activity.

To determine additional chemokines that may be involved in mobilizing human T cells that are antigen-responsive to sites of delayed-type hypersensitivity reactions, we compared the in vitro T lymphocyte chemoattractant effects of the recombinant human (rh) chemokines, MIP-1α, MIP-1β, platelet factor–4 (PF-4), I-309, IL-8, MCAF, transforming growth factor–β (TGF-β), tumor necrosis factor–α (TNF-α), and MGSA to those recombinant human cytokines known to chemoattract T cells. The cytokines were assayed for their chemotactic activity on unstimulated (resting) T cells or T cells that had been activated by incubation with monoclonal antibody to CD3 (anti-CD3) in a 48-well microchemotaxis chamber assay (*3, 5–7*) (Fig. 1).

Activated T cells were stimulated to migrate most by rhMIP-1α, rhMIP-1β, and RANTES, although IL-2 was stimulatory (Fig. 1A) (*4*). Both rhMIP-1α and rhMIP-1β stimulated maximal chemotactic activity at 10 ng/ml, with demonstrable activity at 1 ng/ml (Fig. 1B) (*8*). RANTES was more potent; chemotaxis was maximal at 1 ng/ml. Resting human T cells, in contrast, were not stimulated to migrate by rhMIP-1α or rhMIP-1β, but RANTES was again effective (Fig. 1A). All other tested cytokines had no significant chemoattractant activity on either stimulated or unstimulated T cells over a wide concentration range (*9, 10*). T cell migration to RANTES, rhMIP-1α, and rhMIP-1β was dependent on a concentration gradient (chemotaxis); a checkerboard analysis indicated that <8% of the migration was due to chemokinesis (random migration) (*11*).

T cells consist of phenotypic subpopulations; $CD4^+$ T cells generally have helper activities, and $CD8^+$ T cells have cytotoxic and suppressive activities. To investigate the effects of MIP-1α and MIP-1β on subsets of T lymphocytes, we fractionated peripheral T cells from several normal adult donors into $CD4^+$ or $CD8^+$ T cells (*5, 12*). Activated $CD4^+$ T cells migrated to rhMIP-1β and RANTES, whereas activated $CD8^+$ T cells predominantly migrated in response to rhMIP-1α and RANTES (rhMIP-1β had inconsistent effects on activated $CD8^+$ lymphocytes) (Fig. 2A). Resting $CD4^+$ T cells were only stimulated by RANTES; rhMIP-1α and rhMIP-1β had no significant chemotactic effect on unstimulated $CD4^+$ or $CD8^+$ T cells.

To verify that these chemokines exert direct effects on T lymphocytes, we tested the chemoattractant response of a number of $CD4^+$ T cell clones to these chemokines (*13, 14*). A representative tetanus toxoid (TTx)–reactive T cell clone, 3E12, activat-

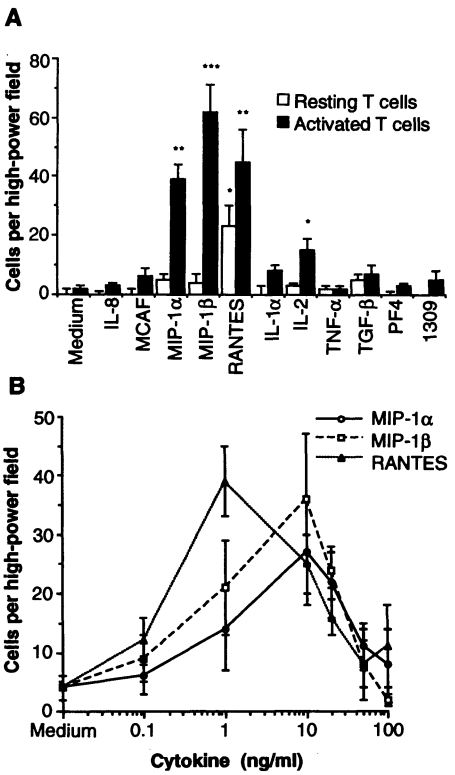

Fig. 1. Human peripheral blood T lymphocyte migration in response to RANTES, rhMIP-1α, and rhMIP-1β. (**A**) Migration of resting and activated T cells in response to RANTES, rhMIP-1α, and rhMIP-1β. Human T lymphocytes (typically >94% pure) were isolated from venous blood and either placed in culture (resting) or stimulated on anti-CD3–coated plates (activated) for 8 hours. Various cytokines were prepared in assay buffer and then added to the lower compartment of a 48-well microchemotaxis chamber. All cytokines used in this experiment were at a concentration of 10 ng/ml, with the exception of rhIL-1α, rhIL-2, rhTNFα, and rhTGFβ, which were all used at 10 U/ml. T cells were then placed in the upper compartment of the chamber and incubated for 3 hours at 37°C. We measured lymphocyte migration by counting the number of cells attached to the lower surface of the filters in five high-power (40×) fields, and each concentration of chemoattractant was tested in triplicate. Results are expressed as the mean number of migrating cells per high-power field (±SD). (**B**) Dose-response curves for activated lymphocyte migration to RANTES, rhMIP-1α, and rhMIP-1β. Points represent various concentrations of chemokine added to the lower wells of a microchemotaxis chamber. Both resting and activated T cells, separated from the chemokines by a polycarbonate filter, were added to the upper compartments of the chamber as described above. Results are expressed as the mean number of migrating cells per high-power field (±SD) from a single representative experiment of 15 performed.

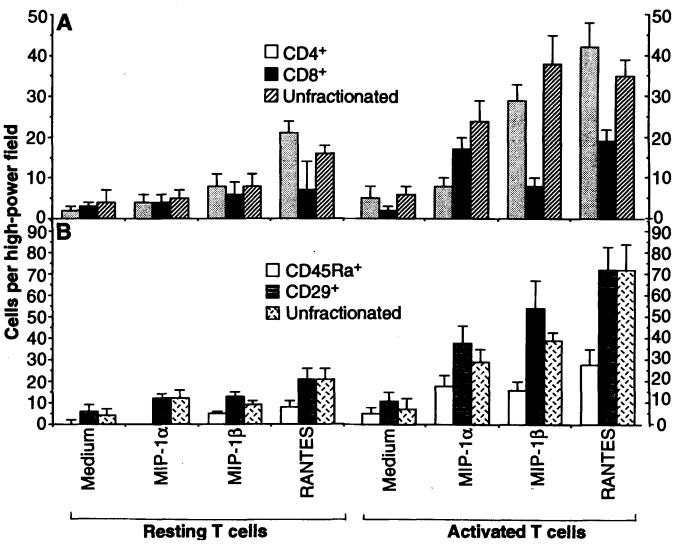

Fig. 2. Phenotype of T lymphocytes migrating to rhMIP-1α, rhMIP-1β, and RANTES. (**A**) Recombinant hMIP-1α and rhMIP-1β selectively attract $CD8^+$ and $CD4^+$ T cells, respectively. Purified resting T cells were separated by negative selection into purified $CD4^+$ (>94%) and $CD8^+$ (>94%) T cell populations (*9*). Both fractionated and unfractionated populations were placed in culture and left unstimulated or activated on anti-CD3–coated plates. After 8 hours of incubation, the cells were harvested and tested for activity in response to various concentrations of rhMIP-1α, rhMIP-1β, RANTES, and MCAF. (**B**) Both rhMIP-1α, rhMIP-1β, and RANTES attract activated $CD45RA^+$ and $CD29^+$ T lymphocytes. With the use of similar purification protocols as described above, highly enriched $CD45RA^+$ (>94%) and $CD29^+$ (>95%) T cell populations were either stimulated on anti-CD3–coated plates or cultured in medium for 6 hours. After incubation, the cells were harvested and tested for chemotactic activity. The results are expressed as the average number of cells counted in five high-powered fields (±SD). Data represent mean values (±SD) of a single experiment of four performed.

ed with either TTx and syngeneic accessory cells or anti-CD3, showed significant T cell migration to rhMIP-1β and RANTES but not to rhMIP-1α (Fig. 3). Of the five human T helper cell clones tested, four exhibited similar patterns of activity; however, one clone was unresponsive to all chemokines tested (*15*). These data support the observation that rhMIP-1β, but not rhMIP-1α, selectively attracts activated $CD4^+$ T cells.

The $CD29^+$ subset of T lymphocytes is generally associated with memory T cell functions and is thought to consist of long-lived effector cells that are preferentially primed to generate immunologically mediated inflammatory responses (*16*). Activated T cells that had been fractionated into naïve ($CD45RA^+$) and memory ($CD29^+$) T cell subsets both migrated to rhMIP-1α, rhMIP-1β, and RANTES (Fig. 2B) (*5, 12*). Unstimulated T cells, whether of the naïve or memory phenotype, did not respond to either rhMIP-1α or rhMIP-1β, but unstimulated memory T cells did migrate in response to RANTES. These results suggest that both the activation status and phenotypic properties of T cell populations determine whether they respond to rhMIP-1α, rhMIP-1β, and RANTES. The increased responsiveness of T cells after activation could be a result of the T cells acquiring new receptors that render them responsive to rhMIP-1α and rhMIP-1β, as demonstrated by the increase in specific binding sites for both MIP-1α and MIP-1β after T cell activation with anti-CD3 and phorbol esters (*9, 17*).

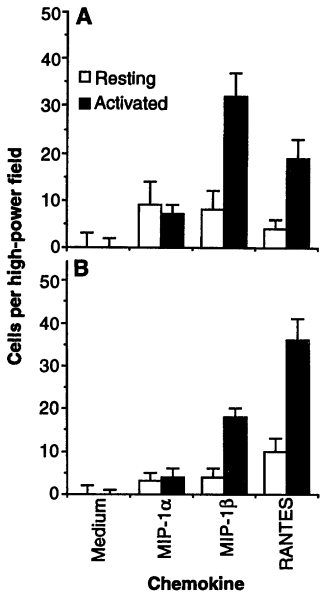

Fig. 3. Recombinant hMIP-1β selectively promotes the locomotion of human $CD4^+$ T cell clones. The human TTx-reactive T cell clone, 3E12, was generated and tested for its ability to respond to various chemokines (*14*). The 3E12 cells were either activated with (**A**) TTx plus syngeneic APCs or (**B**) anti-CD3–coated plates or placed in culture with medium for 8 hours. After incubation, the cells were harvested and tested in a chemotaxis assay with rhMIP-1α, rhMIP-1β, RANTES, or rhMCAF. The results are expressed as the mean number of migrating cells per high-powered field (±SD). Data represent mean values (±SD) of a single experiment of six performed.

Additional experiments were designed to determine whether rhMIP-1α, rhMIP-1β, and RANTES influence the adhesion of T lymphocytes to endothelium. Purified peripheral blood T cell subpopulations were treated with various concentrations of rhMIP-1α, rhMIP-1β, and rhRANTES at 37°C for 6 hours on uncoated or anti-CD3–coated plates. After washing, lymphocytes were incubated with human umbilical cord endothelium pretreated with an optimal concentration of rhIL-1α for the binding assay. All three of these cytokines augment adhesion of stimulated, but not unstimulated, T lymphocytes to IL-1α–treated endothelium (Fig. 4). Once again, MIP-1α preferentially promoted the adherence of $CD8^+$ T lymphocytes, whereas MIP-1β acted on $CD4^+$ lymphocytes. No significant change in T cell adherence was observed with any chemokine on unactivated endothelium or with unstimulated T lymphocytes. Thus, the ability of rhMIP-1α, rhMIP-1β, and RANTES to regulate the adherence of T lymphocytes to vascular endothelium is an important initial step in the migration toward sites of antigenic challenge.

After exposure to antigen, antigen-presenting cells (APC) present in tissues emigrate from the challenged tissue into the draining lymphatic system (*16, 18*). Once within the regional lymph nodes, which are the sites of sensitization, antigen-specific T cells are activated by these migrant APC populations, resulting in the expression of new chemokine receptors (*9, 17*). These antigen-activated T cells then reenter circulation, now capable of directional migration into an inflammatory site. T cell infiltration into the challenged area probably involves a process of sequential endothelial adhesion and then release of T cells, to allow transendothelial passage, followed by adherence to extracellular matrix proteins via integrin molecules (*16, 18*). Our findings lead to the prediction that MIP-1α, MIP-1β, or RANTES participate in attracting the appropriate T cell subsets to an inflammatory site. MIP-1α and MIP-1β may have critical roles in differentially determining the nature of the lymphocyte response to disparate antigenic stimuli. In support of this hypothesis, recent analysis of human T cell migration in a chimeric SCID (severe combined immunodeficiency disease) mouse model reconstituted with human T cells revealed that RANTES and the rhMIP-1 cytokines induce $CD3^+$ T cell infiltration into sites of injection.

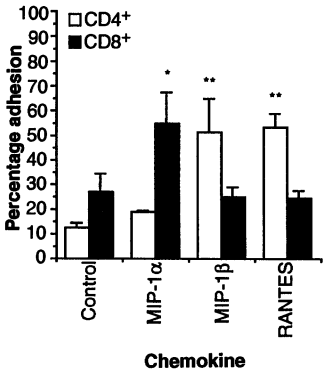

Fig. 4. Recombinant human MIP-1α, MIP-1β, and RANTES induce adhesion of stimulated T cell subsets to IL-1α–treated human umbilical vein endothelium. Both fractionated and unfractionated populations were labeled with ^{51}Cr and preincubated on anti-CD3–coated plates or uncoated plates with optimal concentrations of rhMIP-1α, rhMIP-1β, and rhRANTES for 8 hours at 37°C. After incubation, T cells were added to a rhIL-1α–treated confluent monolayer of human umbilical vein endothelium for 1 hour at 37°C. After washing, bound cpm were measured. Percentage adhesion was calculated according to the following formula: percentage adhesion = measured cpm/[(total cpm − spontaneously released cpm) × 100]. Data represent mean values (±SD) of a single representative experiment of five performed.

REFERENCES AND NOTES

1. J. J. Oppenheim, C. O. C. Zachariae, N. Mukaida, K. Matsushima, *Annu. Rev. Immunol.* **9**, 617 (1991).
2. T. J. Schall, K. Bacon, K. I. Toy, D. V. Goedell, *Nature* **347**, 669 (1990); K. Bacon, K. Westwick, R. D. R. Camp, *Biochem. Biophys. Res. Commun.* **165**, 349 (1989); T. J. Schall, *Cytokine* **3**, 165 (1991).
3. C. G. Larsen, A. O. Anderson, E. Appella, J. J. Oppenheim, K. Matsushima, *Science* **243**, 1464 (1989).
4. H. Kornfeld, J. S. Berman, D. J. Beer, D. M. Center, *J. Immunol.* **134**, 3887 (1985); R. J. Russell, P. C. Wilkinson, F. Sless, D. M. V. Parrott, *Nature* **256**, 646 (1975); P. Miossec, C. Yu, M. Ziff, *J. Immunol.* **133**, 2007 (1984); D. M. Center and W. W. Cruikshank, *ibid.* **128**, 2563 (1983); C. Larsen *et al.*, *Prog. Clin. Biol. Res.* **349**, 419 (1990).
5. K. C. Conlon *et al.*, *J. Immunol.* **149**, 3278 (1992).
6. This procedure routinely yielded a >94% purified T cell subpopulation. For antibody activation of T cells or T cell subsets, the T cell preparations were adjusted to a concentration of 5×10^6 cells/ml and cultured on untreated tissue culture plates or plates previously coated with a 10-μg/ml suspension of anti-CD3 (AMAC, Inc., Westbrook, ME). The T cells were cultured for 6 to 8 hours, harvested, and used in the T cell chemotaxis assay (*3*).
7. A. M. Pilaro, T. J. Sayers, K. L. McCormick, C. W. Reynolds, R. H. Wiltrout, *J. Immunol. Methods* **135**, 213 (1990).
8. Significant donor variation by as much as 50% was observed in the migration of purified T cells and T cell subpopulations to MIP-1α, MIP-1β, and RANTES. Additionally, large clumps of T lymphocytes migrated through the filter. The data are expressed as the number of countable cells per high-power field; however, this is probably a substantial underestimation of the total number of cells that have migrated into or through the filter.

9. D. D. Taub, unpublished data.
10. Neutrophils may be participating in the induction of IL-8–mediated T cell chemotaxis because T cells do migrate in response to IL-8 in neutrophil-depleted rats (*19*). Our lymphocyte preparations may be free of contaminating neutrophils or of other cells that produce such an indirect mechanism of T cell chemotaxis, and therefore lymphocyte attraction in response to IL-8 was not detected.
11. To distinguish between chemotaxis (active and directional cell migration) and chemokinesis (random cell migration), we performed a T cell migration assay in which MIP-1α, MIP-1β, and RANTES were added at equal concentrations on both sides of the collagen-coated polycarbonate filter. Under these conditions, migration was substantially reduced, demonstrating that a concentration gradient is required for active T cell migration to MIP-1α, MIP-1β, and RANTES. Less than 8% of T cell migration to these chemokines could be attributed to chemokinesis.
12. L. A. Turka, J. A. Ladbetter, K. Lee, C. H. June, C. B. Thompson, *J. Immunol.* **144**, 1646 (1990).
13. T. R. Mosmann, H. Cheruruski, M. Bond, M. A. Giedlin, R. L. Coffman, *ibid.* **136**, 2348 (1986).
14. Human tetanus toxoid– and diptheria toxoid–reactive CD4+ T cell clones and cell lines were established in our laboratory with standard cloning procedures (*12*). Several clones were isolated and were found to be CD4+ and CD29+ T cells with a T helper cell–1–like lymphokine profile. We maintained these cells on a 15- to 21-day stimulation schedule by using irradiated autologous peripheral blood mononuclear cells and antigen (2 μg/ml) and rhIL-2 (25 U/ml). Before use, T cell clones were passaged over a Ficoll-Hypaque gradient to enrich for viable cells.
15. Several TTx- and diptheria toxoid (DTx)–reactive T cell clones were tested for chemotactic activity. Four CD4+ TTx-reactive clones migrated to rhMIP-1β and RANTES but not to rhMIP-1α. One DTx-reactive CD4+ T cell clone did not respond to rhMIP-1α, rhMIP-1β, and RANTES. Additionally, several long-term nonclonal TTx- and DTx-reactive CD4+ T cell lines demonstrated a preferential migration to MIP-1β and not to MIP-1α. CD8+ alloreactive T cell lines and clones demonstrated variable responses to both MIP-1 compounds.
16. S. Aiba and S. I. Katz, *J. Immunol.* **145**, 2791 (1990); S. E. Macatonia, A. J. Edwards, S. C. Knight, *Immunology* **59**, 509 (1986); S. E. Macatonia, S. C. Knight, A. J. Edwards, S. Griffiths, P. Fryer, *J. Exp. Med.* **166**, 1654 (1987); J. Silberberg-Sinakin, G. J. Thorbecke, R. L. Baer, S. A. Rosenthal, V. Berezowsky, *Cell. Immunol.* **25**, 137 (1976); C. P. Larsen *et al.*, *J. Exp. Med.* **172**, 1483 (1990).
17. M. Napolitano, K. B. Seaman, W. Leonard, *J. Exp. Med.* **173**, 285 (1990); K. O. Oh *et al.*, *J. Immunol.* **147**, 2978 (1991).
18. T. A. Springer, *Nature* **346**, 425 (1990); M. L. Dustin and T. A. Springer, *Annu. Rev. Immunol.* **9**, 27 (1991); M. L. Dustin and T. A. Springer, *Nature* **341**, 619 (1989); Y. Shimizu, G. Van Seventer, K. J. Horgan, S. Shaw, *ibid.* **345**, 250 (1990); R. O. Hynes, *Cell* **69**, 11 (1992).
19. C. Kudo, A. Araki, K. Matsushima, F. Sendo, *J. Immunol.* **147**, 2196 (1991).
20. We thank M. Yamada (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan), Y. Iriye (Otsuka Corp., Rockville, MD), M. Tsang (R&D Systems, Minneapolis, MN), W. Leonard (National Heart, Lung, and Blood Institute, NIH, Bethesda, MD), M. S. Krangel (Duke University Medical School, Durham, NC), B. Sherry (Picower Institute, Manhasset, NY), G. Wong (Genentech Co., San Francisco, CA), and L. Ellingsworth (Collagen Corp., Palo Alto, CA). We also thank D. Longo, W. Murphy, J. O'Shea, S. Durum, W. Leonard, and J.-M. Wang for helpful criticisms. Supported in part by the Department of Health and Human Services under contract N01-C0-74102.

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Requirement for Tyrosine Kinase p56^{lck} for Thymic Development of Transgenic γδ T Cells

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The Src-related protein tyrosine kinase p56^{lck} is essential for antigen-specific signal transduction and thymic maturation of T cells that have an αβ T cell receptor (TCR), presumably by physical association with CD4 or CD8 molecules. To evaluate the requirement for p56^{lck} in the development of T cells that have γδ TCRs, which generally do not express CD4 or CD8, p56^{lck} mutant mice were bred with TCRγδ transgenic mice. Few peripheral cells that carried the transgenes could be detected in p56$^{lck-/-}$ mice, although 70 percent of thymocytes were transgenic. Development of transgenic γδ$^+$ thymocytes was blocked at an early stage, defined by interleukin-2 receptor α expression. However, extrathymic development of CD8αα$^+$TCRγδ$^+$ intestinal intraepithelial lymphocytes appeared to be normal. Thus, p56^{lck} is crucial for the thymic, but not intestinal, maturation of γδ T cells and may function in thymic development independently of CD4 or CD8.

The molecular requirements for the development of TCRγδ$^+$ lymphocytes are poorly characterized (*1*). Thymic development of TCRαβ cells unfolds in stages defined by expression of CD4 and CD8 (*2*). Early CD4$^-$CD8$^-$ precursors become CD4$^+$CD8$^+$ cells and finally mature into CD4$^+$ or CD8$^+$ single-positive cells. Specific recognition of self major histocompatibility complex (MHC) molecules by the TCRαβ$^+$ thymocytes is the prerequisite for thymic selection events and maturation to single-positive lymphocytes (*2*). Besides the specific antigen receptor, CD4 and CD8 molecules are crucial for thymic selection and maturation of TCRαβ$^+$ lymphocytes (*3*).

Antigen-specific stimulation of T cells results in the activation of protein tyrosine kinases and subsequent tyrosine phosphorylation of several intracellular substrates (*4*). The Src-related protein tyrosine kinase p56^{lck} (*5*) is essential for antigen-induced signal transduction mediated by TCRαβ-CD3 complexes (*6*). Because p56^{lck} is physically associated with the cytoplasmic portions of CD4 or CD8 molecules (*7*) expressed on TCRαβ$^+$ cells, activation of αβ$^+$ T cells is probably modulated by CD4 or CD8 molecules by means of p56^{lck} (*8*); mice rendered deficient for p56^{lck} by homologous recombination have a block in thymic development of TCRαβ$^+$ cells and few TCRαβ$^+$ cells are present in the lymph nodes and spleens of these mice (the number of TCRαβ$^+$ cells in p56$^{lck-/-}$ mice is 5 to 15% of that in p56$^{lck+/-}$ and p56$^{lck+/+}$ controls) (Fig. 1) (*9*). However, the role of p56^{lck} in signal transduction and in the development of TCRγδ$^+$ lymphocytes, which generally do not express CD4 or CD8 accessory molecules, is not known (*1*).

To evaluate the requirement for p56^{lck} in the development of γδ$^+$ T cells, we crossed the p56^{lck} mutation (*9*) into mice that expressed a transgenic (tg) γδ TCR (V_α11.3J_δ1C_δ/V_γ2J_γ1C_γ1) (*10, 11*). In these mice, approximately 70% of peripheral T cells express the tg γδ TCR, as assessed by staining with a fluoresceinated monoclonal antibody (mAb) to V_γ2 (Fig. 1) (*11, 12*). The total number of T lymphocytes in the lymph nodes and spleens of tg$^{+/+}$p56$^{lck+/+}$ mice was equivalent to the numbers found in tg$^{-/-}$p56$^{lck+/+}$ wild-type mice. The total number of T cells in the spleen and lymph nodes of heterozygous tg$^{+/-}$p56$^{lck+/-}$ mice was similar to that in tg$^{+/+}$p56$^{lck+/+}$ mice, although the number of cells that expressed V_γ2 was slightly lower (~60% of the total number of T cells).

In contrast to p56$^{lck+/+}$ and p56$^{lck+/-}$ mice, $V_\gamma2^+$ T lymphocytes were nearly absent from the spleen, blood, and lymph nodes of tg$^{+/-}$p56$^{lck-/-}$ mice, which thus resembled tg$^-$p56$^{lck-/-}$ animals (Fig. 1). In addition, the total peripheral T cell number in tg$^{+/-}$p56$^{lck-/-}$ and tg$^{-/-}$p56$^{lck-/-}$ mice was reduced as described (Fig. 1) (*9*). Thus, tg $V_\gamma2^+$ lymphocytes were absent in the periphery of p56^{lck}-deficient mice, and the presence of rearranged TCRγδ transgenes could not restore normal numbers of peripheral T cells.

The V_α11V_γ2 TCR expressed in these tg mice was originally cloned from a BALB/c

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