clonal selection, the presence of two distinct TCRs on the same cell should have limited consequences. Because the signal to switch off the thymocyte recombination machinery seems to be closely dependent on an interaction of the surface TCR with a specific thymic ligand during development (*12*), lack of genotypic exclusion of TCRα and TCRγ chain genes should allow a given T cell to try several TCR chains until it expresses a functionally relevant (that is, selectable) TCR. Thus, if two surface α or γ chains are expressed on the lymphocyte surface, one of these receptors, the one that was not selected for, should have little chance to encounter its nominal ligand in the periphery. However, many TCRs also have affinity for superantigens, which bind germline V-specific residues (*13*) and hence should be efficiently recognized by both selected and nonselected TCRs. In this context, the probability that a lymphocyte expressing two surface TCRs would encounter ligands for each of its expressed Ag receptors becomes more significant. If one of these ligands is an autoantigen, one could imagine that such cells might initiate an autoimmune reaction.

REFERENCES AND NOTES

1. F. W. Alt, T. K. Blackwell, G. D. Yancopoulos, *Science* **238**, 1079 (1987); D. Weaver, F. Constantini, T. Imanishi-Kari, D. Baltimore, *Cell* **42**, 117 (1985); Y. Uematsu *et al.*, *ibid.* **52**, 831 (1988).
2. M. Malissen *et al.*, *Immunol. Today* **13**, 315 (1992).
3. M. Malissen *et al.*, *Cell* **52**, 49 (1988).
4. J. S. Heilig and S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8070 (1987).
5. F. Koning *et al.*, *J. Immunol.* **141**, 2057 (1988); A. Ezquerra *et al.*, *Eur. J. Immunol.* **22**, 491 (1992).
6. F. Davodeau, M.-A. Peyrat, M.-M. Hallet, M. Bonneville, unpublished results.
7. Among ten clones expressing $V_\gamma9$ chains only, linear MFI values obtained with 7B6 mAb were strictly correlated with MFI values obtained with 510 (pan-δ) mAb ($r = 0.96$, $P < 0.001$). We obtained similar results with cells expressing $V_\gamma4$ chains only, using 4A11 mAb instead of 7B6 mAb ($r = 0.94$, $P < 0.001$). In contrast, analysis of $4A11^+,7B6^+$ T cell clones demonstrated a lack of correlation between MFI values obtained with either $V_\gamma4$- or $V_\gamma9$-specific mAb versus pan-δ mAb. However, a strong correlation was observed between cumulated linear MFI values obtained with each V_γ-specific mAb versus pan-δ mAb ($r = 0.96$, $P < 0.001$). Accordingly, staining intensities obtained with V-specific mAb relative to pan-δ were inversely correlated ($r = -0.95$, $P < 0.001$) (Fig. 1C). Taken together, this analysis strongly suggests that 7B6 and 4A11 mAbs recognized distinct γ chains on the surface of $7B6^+,4A11^+$ clones.
8. D. R. Littman *et al.*, *Nature* **326**, 85 (1987); M. P. Lefranc, A. Forster, T. H. Rabbitts, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9596 (1986).
9. J. Borst *et al.*, *Eur. J. Immunol.* **19**, 1559 (1989).
10. $4A11^+,7B6^+$ T cell clones were analyzed by indirect immunofluorescence with the $V_\delta1$-specific mAb A13 [S. Ferrini *et al.*, *Eur. J. Immunol.* **19**, 57 (1989)], the $V_\delta2$-specific mAb 389 (*14*), and mAb 515, which recognizes all δ chains except those comprising the $V_\delta1$ region (I. Houde *et al.*, in preparation). Clones B1, B4, and 2B55 were $A13^+,389^-,515^-$, clones A4, B22, and D5 were $A13^-,389^+,515^+$, and clones C7 and B16 were $A13^-,389^-,515^+$ (M.-A. Peyrat and M. Bonneville, unpublished results). Because none of the $V_\delta1^+$ T cell clones (B1, B4, or 2B55) bound 515 mAb, they expressed δ chains comprising the $V_\delta1$ region exclusively. Because direct sequencing of polymerase chain reaction (PCR) products amplified from cDNA of clones with $V_\delta1$ and C_δ primers yielded a single in-frame sequence (Fig. 2), a single δ chain paired with either the $V_\gamma9$ or the $V_\gamma4$ chains was expressed on their surface.
11. Expressed TCRγ genes were sequenced by PCR in a panel of 28 randomly chosen T cell clones. Among the clones, three expressed two distinct productive γ transcripts ($V_\gamma8J_\gamma2C_\gamma2$ and $V_\gamma8J_\gamma2C_\gamma2$; $V_\gamma4J_\gamma1C_\gamma1$ and $V_\gamma4J_\gamma P1C_\gamma1$; and $V_\gamma4J_\gamma2C_\gamma2$ and $V_\gamma9J_\gamma PC_\gamma1$) (F. Davodeau, M.-M. Hallet, M. Bonneville, unpublished results).
12. L. A. Turka *et al.*, *Science* **253**, 778 (1991); P. Borgulya, H. Kishi, Y. Uematsu, H. Von Boehmer, *Cell* **69**, 529 (1992).
13. P. Marrack and J. Kappler, *Science* **248**, 705 (1990).
14. F. Davodeau *et al.*, *J. Biol. Chem.*, in press.
15. H. Vié *et al.*, *Blood* **74**, 285 (1989).
16. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
17. M. P. Lefranc and T. H. Rabbitts, *Res. Immunol.* **141**, 267 (1990).
18. D. Kabelitz, *Crit. Rev. Immunol.* **11**, 251 (1992).
19. S. Jitsukawa, S. Faure, N. Lipinski, F. Triebel, T. Hercend, *J. Exp. Med.* **166**, 1192 (1987).
20. M.-P. Lefranc and T. H. Rabbitts, *Nature* **316**, 464 (1985).
21. T. Boehm *et al.*, *EMBO J.* **7**, 385 (1988).
22. M.-P. Lefranc, A. Forster, T. H. Rabbitts, *Nature* **319**, 420 (1986); M.-P. Lefranc, A. Forster, R. Baer, M. A. Stinson, T. H. Rabbitts, *Cell* **45**, 237 (1986).
23. Y. Takihara *et al.*, *J. Exp. Med.* **169**, 393 (1989).
24. G. De Libero *et al.*, *ibid.* **173**, 1311 (1991).
25. K. Maeda *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6536 (1987).
26. F. Davodeau *et al.*, unpublished data.

27. We thank F. Romagné, D. Kabelitz, and T. Hercend for providing mAbs and W. Laird, P. A. Cazenave, C. A. Janeway, Jr., A. Lanzavecchia, and B. Malissen for critical reading of the manuscript and helpful comments. Supported in part by a grant from the Association pour la recherche contre le Cancer.

18 February 1993; accepted 13 May 1993

Switch of CD8 T Cells to Noncytolytic $CD8^-CD4^-$ Cells That Make T_H2 Cytokines and Help B Cells

François Erard,* Marie-Thérèse Wild, Jose A. Garcia-Sanz, Graham Le Gros

CD8⁺ T cells are a major defense against viral infections and intracellular parasites. Their production of interferon-γ (IFN-γ) and their cytolytic activity are key elements in the immune response to these pathogens. Mature mouse CD8⁺ T cells that were activated in the presence of interleukin-4 (IL-4) developed into a $CD8^-CD4^-$ population that was not cytolytic and did not produce IFN-γ. However, these $CD8^-$ cells produced large amounts of IL-4, IL-5, and IL-10 and helped activate resting B cells. Thus, CD8 effector functions are potentially diverse and could be exploited by infectious agents that switch off host protective cytolytic responses.

The activation of resting $CD4^+$ T cells by antigens in the presence of IL-4 directs their development toward the production of IL-4, IL-5, and IL-10 cytokines (T_H2 phenotype) (*1*). We sought to establish whether the phenotype of CD8 cells could also be influenced by IL-4. The basic experimental outline consisted of activating $CD8^+$ cells (sorted with a fluorescence-activated cell sorter) for 6 days in in vitro primary cultures in the presence and absence of IL-4 and then analyzing the resulting phenotype with respect to surface markers and expression of functional activities. The low frequency of antigen-specific CD8 cells made the use of antigen-stimulated primary culture systems impractical. Therefore, we used a primary culture system activated by a mitogen [phorbol 12-myristate 13-acetate (PMA) + ionomycin + IL-2] to ensure a high efficiency of CD8 cell activation, confirmed by limiting dilution analysis (*2*). Small numbers of $CD8^+$ cells (800 cells per well) were seeded in the mitogen-activated primary cultures because this ensured that the activation and proliferation of the CD8 cells were independent of accessory cells (*2*).

Fluorescence flow cytometry–sorted $CD8^+$ cells (>99% purity) were stimulated to grow in primary cultures that contained different combinations of PMA, ionomycin, IL-2, and IL-4 (*3*). After 6 days of culture, the cells were stained for the expression of the αβ T cell receptor (TCR), Thy-1.2, CD2, CD4, CD8, CD44 (Pgp1), and CD45 (T200) surface markers. All cells (99%) from each primary culture condition expressed TCRαβ and Thy-1.2, and expression was not affected either by activation or by the addition of IL-4 (Fig. 1). After activation, CD44 expression was increased to a similar degree in each culture condition. However, CD8 completely disappeared from cells cultured with PMA,

F. Erard, M.-T. Wild, G. Le Gros, Department of Allergy/Immunology, Pharmaceuticals Research Division, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland.
J. A. Garcia-Sanz, Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basel, Switzerland.

*To whom correspondence should be addressed.

ionomycin, IL-2, and IL-4 (Fig. 1A) but was normally expressed on cells from all the other primary culture conditions. The effect of IL-4 on CD8 expression was dependent on the presence of ionomycin because cultures treated with PMA, IL-2, and IL-4 were positive for CD8. Northern (RNA) blot mRNA analysis revealed that the loss of surface CD8 correlated with a decrease of mRNA encoding the CD8α chain (Fig. 1B).

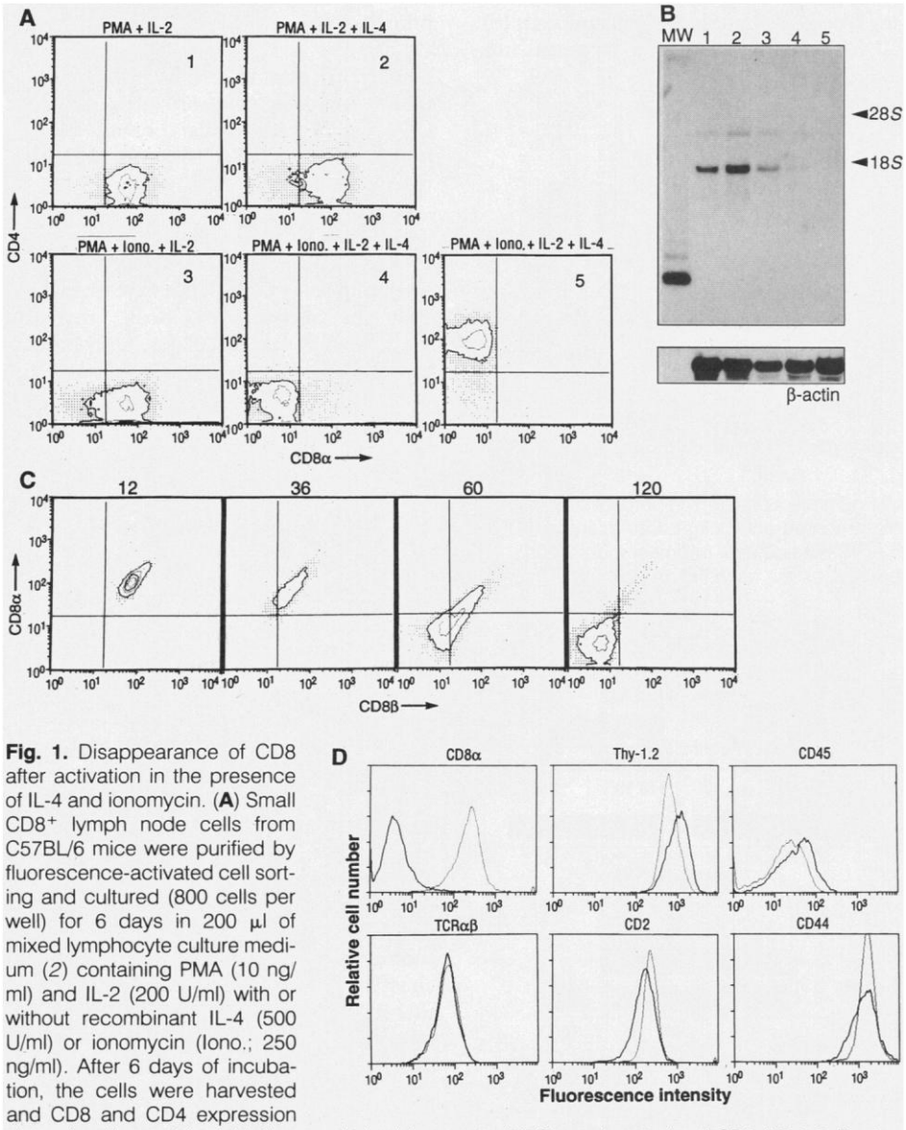

Fig. 1. Disappearance of CD8 after activation in the presence of IL-4 and ionomycin. (**A**) Small $CD8^+$ lymph node cells from C57BL/6 mice were purified by fluorescence-activated cell sorting and cultured (800 cells per well) for 6 days in 200 μl of mixed lymphocyte culture medium (*2*) containing PMA (10 ng/ml) and IL-2 (200 U/ml) with or without recombinant IL-4 (500 U/ml) or ionomycin (Iono.; 250 ng/ml). After 6 days of incubation, the cells were harvested and CD8 and CD4 expression determined by staining with fluorescein isothiocyanate (FITC)–conjugated anti-CD8 (53-6.7, Becton Dickinson, San Jose, California) and phycoerythrin (PE)-labeled anti-CD4 (GK1.5, Becton Dickinson) and flow cytometry analysis (panels 1 to 4). $CD4^+$ cells were prepared as above, cultured in PMA, ionomycin, IL-2, and IL-4, and analyzed for CD4 and CD8 expression (panel 5). Cell proliferation was followed for each culture condition and was similar, regardless of the presence or absence of IL-4 or ionomycin (*3*). (**B**) Total cellular RNA was prepared from cells cultured as described in panels 1 to 5 of (A) (here, corresponding to lanes 1 through 5) by the guanidinium isothiocyanate–$CsCl_2$ method. RNA was size-fractionated in 1.2% formaldehyde-agarose gel electrophoresis (10 μg per lane) and subjected to Northern blot analysis with a ^{32}P-labeled Pst I–Xba I fragment of the α5 clone coding for CD8α complementary DNA (cDNA). For control purposes, the blot was washed and reprobed with the 1.1-kb Pst I–Pst I fragment of the mouse β-actin cDNA clone pAL 41 (*17*). MW, molecular weight markers. (**C**) Time course (in hours) for the disappearance of CD8α and CD8β chains from the surface of CD8 cells. $CD8^+$ cells were cultured with PMA, ionomycin, IL-2, and IL-4, and the expression of CD8α and CD8β chains was followed for 5 days by staining with PE-labeled 53-6.7 (Pharmingen, San Diego, California) and FITC-labeled 53-5.8 (Pharmingen) antibodies, respectively, and flow cytometry analysis (Becton Dickinson). (**D**) Phenotypic characterization of the $CD8^-CD4^-$ subset. The broken lines represent treatment with PMA and IL-2; the solid lines show treatment with PMA, ionomycin, IL-2, and IL-4. The cells were stained with a panel of fluorescent antibodies (Pharmingen) and flow cytometry analysis.

The $CD8^-$ phenotype was not a result of the expansion of $CD4^+$ cells; none were detected in any of the primary cultures. Also, the $CD8^-CD4^-$ cells were not derived from contaminating $CD4^+$ cells because sorted $CD4^+$ cells cultured with PMA, ionomycin, IL-2, and IL-4 did not lose expression of the CD4 molecule (Fig. 1A).

A kinetics study indicated that surface expression of CD8α and CD8β chains both decreased gradually on all cells over a 5-day culture period (Fig. 1C). This, together with the observation that the cultures either containing or not containing IL-4 had equivalent doubling times (*3*), indicated that the $CD8^-$ cells were derived from activated $CD8^+$ cells and did not represent the outgrowth of a minor $CD8^-$ subpopulation. The $CD8^-$ phenotype appeared to be stable because reexpression of CD8 did not occur when $CD8^-$ cells were cultured for an additional 6 days in the absence of IL-4. Therefore, activated, proliferating $CD8^+$ T cells could be induced by IL-4 to undergo a phenotype switch to a $CD8^-$ cell that still expressed Thy-1.2, TCRαβ, CD2, CD44, or CD45 (Fig. 1D).

To identify the functional potential of the IL-4–cultured $CD8^-$ cells, we analyzed cytolytic activity on lectin-coated P815 target cells (Fig. 2A). The $CD8^-$ population from cultures treated with PMA, ionomycin, IL-2, and IL-4 had no detectable lytic activity, whereas $CD8^+$ cells from the primary cultures treated with either PMA and IL-2 or PMA, IL-2, and IL-4 lysed the target cells (Fig. 2A). The absence of CD8 expression was not responsible for the decrease in lytic activity because cells cultured in PMA, ionomycin, and IL-2 had normal expression of CD8 but also had a low lytic response. The lack of lytic activity could be correlated with the absence of perforin mRNA induction (Fig. 2B) (*4*). Northern blot analysis (quantified by phospho-imaging) indicated that perforin mRNA expression was reduced by 95% in the nonlytic $CD8^-$ population compared to cells activated with PMA and IL-2. The $CD8^+$ cells from cultures treated with PMA, ionomycin, and IL-2 that also had reduced lytic activity had an 80% reduction in perforin mRNA expression. As expected, $CD4^+$ cells activated under identical conditions expressed no detectable perforin mRNA. Thus, IL-4 and ionomycin can control the induction of the lytic machinery at the mRNA level.

To determine whether the IL-4–cultured CD8 cells had gained other T cell functions, we assayed cytokine production after restimulation of the CD8 cells for 24 hours with plate-bound antibody to CD3 (anti-CD3) (*1*). The cytokine pattern of the CD8 cells cultured in PMA and IL-2 (5), PMA, IL-2, and IL-4 (5), or PMA, ionomycin, and IL-2 (Fig. 3A) was consistent with

previous work (6): production of large amounts of IFN-γ (750 U/ml for 10^5 cells) but no IL-4, IL-5, or IL-10 detected. The $CD8^-$ population from cultures treated with PMA, ionomycin, IL-2, and IL-4, however, produced a concentration of IFN-γ of only 90 U/ml for 10^5 cells, although it produced 2000 U/ml of IL-4, 9000 U/ml of IL-5, and 5000 U/ml of IL-10 (Fig. 3). This $CD8^-$ cell therefore represents a T cell subset that can produce what is normally considered the T_H2 pattern of cytokines (*7*).

We tested whether these cells could provide the necessary signals for the in vitro activation and differentiation of small resting B cells into antibody-producing cells (*8*). $CD8^-$ or $CD8^+$ cells were harvested from primary cultures, washed, restimulated on plates coated with anti-CD3, and cocultured with 10^4 purified splenic B cells from *nu/nu* mice in the presence of IL-4 for 7 days. The stimulated $CD8^-$ cells induced B cells to produce immunoglobulin G1 (IgG1) (300 to 400 ng/ml), whereas the $CD8^+$ cells from primary cultures treated with PMA and IL-2 (*9*), PMA, IL-2, and IL-4 (*9*), or PMA, ionomycin, and IL-2 (Fig. 3B) failed to induce any detectable antibody production. $CD4^+$ cells from similar primary cultures induced B cells to produce comparable quantities of antibodies.

We investigated whether other conditions of CD8 cell activation would allow IL-4 to induce the $CD8^-$ phenotype. Primary cultures of $CD8^+$ cells were established with the allogeneic $TCR\alpha\beta^-$ thymoma BW5147 (*10*) as a stimulator. Only primary cultures containing allogeneic cells and IL-4 produced a noncytolytic (*11*) $CD8^-$ subpopulation that represented about 25% of the activated cells (Fig. 4). Staining with antibodies to the TCR showed that these $CD8^-$ cells were not contaminated with BW5147 stimulator cells (*10*).

The cell type described here could enable a host to mount a class I–restricted "T_H2-like" immune response. Still other CD8 phenotypes can be induced in this culture system: the addition of transforming growth factor–β (TGF-β) to cultures treated with PMA, ionomycin, and IL-2 led to a $CD8^+$ phenotype that was noncytolytic, produced no cytokines, and could not help B cells. If other T cell stimuli (anti-CD3) were combined with IL-4 in culture, cytolytic $CD8^+$ phenotypes would develop that can produce IL-4, IL-5, and IL-10 and help B cells. Our data therefore indicate that CD8 effector cells can be divided into subsets with a potential for heterogeneity in class I–restricted CD8 immune responses that may be elicited by infectious agents or foreign stimuli.

Our findings describe an IL-4–dependent mechanism where CD8 cytolytic responses can be turned into IL-4, IL-5, and IL-10 cytokine responses. In situations where the CD8 cytolytic activity is protective, the switch to IL-4, IL-5, and IL-10 production would probably allow a pathogen to escape elimination. In this regard, it is significant that *Leishmania major* infection is fatal in strains of mice that readily produce IL-4 (*12*), possibly because of an IL-4–induced change in the protective CD8 and CD4 immune response (*13*). IL-4–producing CD8 cells have also been implicated in the pathology of leprosy (*14*). Also, the phenomenon of intense IL-4–IL-5 cytokine activity that precedes the onset of acquired immunodeficiency syndrome (AIDS) may reflect the switching of CD8 cells from a cytolytic phenotype to the

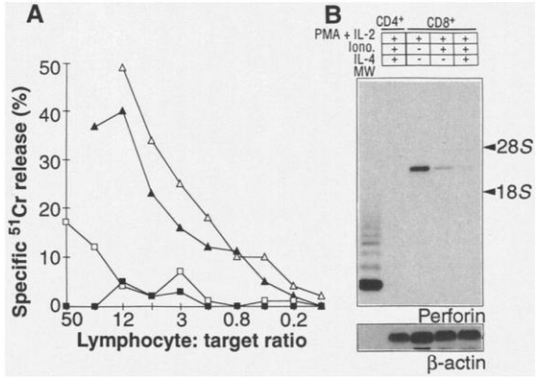

Fig. 2. Inhibition of CD8 cell cytolytic activity and perforin mRNA expression by culture in the presence of ionomycin and IL-4. (**A**) $CD8^+$ cells were prepared and cultured as in Fig. 1. ▲, Treatment with PMA and IL-2; △, treatment with PMA, IL-2, and IL-4; □, treatment with PMA, ionomycin, and IL-2; ■, treatment with PMA, ionomycin, IL-2, and IL-4. After 6 days, the cells were harvested and tested against 1000 ^{51}Cr-labeled P815 target cells in the presence of concanavalin A (2 μg/ml) in microtiter plates in a final volume of 200 μl. After incubation at 37°C for 3.5 hours, the plates were centrifuged and 100 μl was removed for gamma counting. The percent specific lysis was calculated (*18*). (**B**) $CD8^+$ and $CD4^+$ cells were prepared and cultured as indicated. RNA, prepared as described in Fig. 1, was hybridized with a ^{32}P-labeled Pst I fragment (nucleotides 143 to 1289) of the perforin (*19*) cDNA and β-actin. Abbreviations are as in Fig. 1.

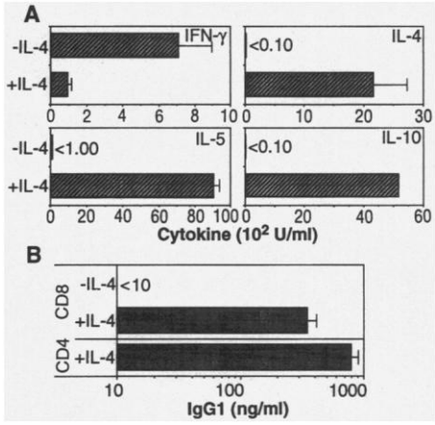

Fig. 3. Cytokine secretion and B cell help by cultured CD8 cells. Small $CD8^+$ lymph node cells from BALB/c mice were cultured for 6 days with PMA, ionomycin, and IL-2 either with or without IL-4. Cells were harvested from each culture, washed, and recultured (10^5 cells per 200 μl) on plates coated with a monoclonal antibody to CD3ε (20 μg/ml) in the presence of IL-2 for 24 hours (*1*). (**A**) The supernatants were assayed for the cytokines IL-4, IL-5, IL-10, and IFN-γ by enzyme-linked immunosorbent assay (ELISA) with antibodies (Pharmingen) and standardized to commercial cytokines (Genzyme) (*1*). The results shown are the mean of two independent cultures ± SE. (**B**) The cultures were irradiated and cocultured with 10^4 Percoll-purified B cells from *nu/nu* mice in the presence of IL-4 for 7 days. Immunoglobulin G1 (IgG1) antibody production was quantified by ELISA with plates coated with goat antibody to mouse IgG1 (5 μg/ml) (Southern Biotechnology Associates, Birmingham, Alabama). Bound IgG1 was detected with alkaline phosphatase–coupled goat antibody to mouse IgG1 and compared to standards. For comparison, similar experiments were performed with CD4 cells. The results are the mean of two independent cultures ± SE.

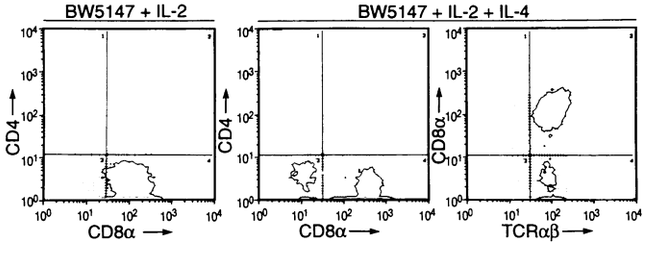

Fig. 4. Effect of IL-4 on the phenotype of alloantigen-stimulated $CD8^+$ cells. Three thousand BALB/c ($H\text{-}2^d$) $CD8^+$ lymph node cells were cultured in the presence of 5000 irradiated (50 gray) BW5147 ($H\text{-}2^k$) thymoma cells deficient in $TCR\alpha\beta$ expression (*10*) and IL-2 (200 U/ml) and with or without IL-4 (500 U/ml). Cells were cultured for 9 days, harvested, and analyzed for the expression of CD4 (GK1.5, Becton Dickinson), CD8 (53-6.7, Becton Dickinson), and $TCR\alpha\beta$ (H57-597, Pharmingen).

CD8$^-$ subset that produces IL-4 and IL-5 (*15*). The observation that a proportion of alloreactive CD8 cells can be diverted by IL-4 from the normal cytolytic CD8 phenotype suggests a possible therapy in the area of transplant rejection. Potentially graft-destructive CD8 lytic responses may be able to be turned into more tolerable or appropriate lymphokine responses by the administration of IL-4 (*16*).

REFERENCES AND NOTES

1. G. Le Gros, S. Z. Ben-Sasson, R. Seder, F. D. Finkelman, W. E. Paul, *J. Exp. Med.* **172**, 921 (1990); S. L. Swain, A. D. Weinberg, M. English, G. Huston, *J. Immunol.* **145**, 3796 (1990); M. Kopf *et al.*, *Nature* **362**, 245 (1993).
2. F. Erard, M. Nabholz, A. Dupuy-d'Angeac, H. R. MacDonald, *J. Exp. Med.* **162**, 1738 (1985); F. Erard *et al.*, *J. Immunol.* **134**, 1644 (1985).
3. On day 6 of culture, approximately 3×10^5 to 5×10^5 cells/ml were recovered from all culture conditions, which indicates a doubling time of 14 to 15 hours. Purified CD4$^+$ lymph node cells (1000 cells per well) cultured in the presence of PMA, ionomycin, IL-2, and IL-4 achieved concentrations of 3×10^5 to 7×10^5 cells/ml by day 6.
4. Resting CD8$^+$ cells do not express perforin [E. R. Podack, H. Hengartner, M. G. Lichtenheld, *Annu. Rev. Immunol.* **9**, 129 (1991); A. Zlotnik, D. I. Godfrey, M. Fischer, T. Suda, *J. Immunol.* **149**, 1211 (1992)].
5. Cytokine production by CD8 cells from cultures treated for 6 days with PMA and IL-2 or PMA, IL-2, and IL-4 was identical to cytokine production by CD8 cells from cultures treated with PMA, ionomycin, and IL-2.
6. P. A. Geuerne, P. F. Piguet, P. Vassalli, *J. Immunol.* **130**, 2225 (1983); A. Kelso and A. Glasebrook, *ibid.* **132**, 2924 (1984).
7. T. R. Mosmann, H. Cherwinski, M. W. Bond, M. A. Giedlin, R. L. Coffman, *ibid.* **136**, 2348 (1986).
8. T. Owen, *Eur. J. Immunol.* **18**, 395 (1988); R. J. Noelle and E. C. Snow, *Immunol. Today* **11**, 361 (1990).
9. No IgG1 production by B cells was induced by CD8 cells from cultures treated with PMA and IL-2 or PMA, IL-2, and IL-4.
10. J. White *et al.*, *J. Immunol.* **143**, 1822 (1989).
11. Sorted CD8$^-$ cells had no detectable lytic activity (<2%) against allogeneic targets (BW5147 cells) at an effector:target ratio of 12:1, whereas CD8$^+$ cells had easily detectable activity (28%).
12. F. P. Heinzel, M. Sadick, S. S. Mutha, R. Locksley, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7011 (1991).
13. R. G. Titus *et al.*, *Eur. J. Immunol.* **17**, 1429 (1987); I. Muller, T. H. Pedrazzini, P. Kropf, J. A. Louis, G. Milon, *Int. Immunol.* **3**, 587 (1991).
14. P. Salgame *et al.*, *Science* **254**, 279 (1991).
15. J. B. Margolick *et al.*, *Clin. Immunol. Immunopathol.* **51**, 348 (1989); E. Maggi *et al.*, *Eur. J. Immunol.* **17**, 1685 (1987); G. M. Shearer and M. Clerici, *Prog. Chem. Immunol.* **54**, 21 (1992); A. Sher *et al.*, *Immunol. Rev.* **127**, 183 (1992).
16. V. S. Abraham, D. Sachs, M. Sykes, *J. Immunol.* **148**, 3746 (1992).
17. S. Alonso, A. Minty, Y. Bourlet, M. Buckingham, *J. Mol. Evol.* **23**, 11 (1986).
18. J.-C. Cerottini, H. D. Engers, H. R. MacDonald, K. T. Brunner, *J. Exp. Med.* **140**, 703 (1974).
19. J. A. Garcia-Sanz and E. R. Podack, *Eur. J. Immunol.*, in press.

20. We thank M. Wesp and A. Kiefer for flow cytometry, S. Walti for technical assistance, B. Pfeiffer for artwork, Dr. P. Cosson for the CD8α cDNA probe, and F. Ronchese, T. Staehelin, C. Heusser, and R. Jessberger for critical review of the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffmann–La Roche Ltd.

1 February 1993; accepted 13 May 1993

LIFRβ and gp130 as Heterodimerizing Signal Transducers of the Tripartite CNTF Receptor

Samuel Davis,* Thomas H. Aldrich, Neil Stahl, Li Pan, Tetsuya Taga, Tadamitsu Kishimoto, Nancy Y. Ip, George D. Yancopoulos*

The ciliary neurotrophic factor (CNTF) receptor complex is shown here to include the CNTF binding protein (CNTFRα) as well as the components of the leukemia inhibitory factor (LIF) receptor, LIFRβ (the LIF binding protein) and gp130 [the signal transducer of interleukin-6 (IL-6)]. Thus, the conversion of a bipartite LIF receptor into a tripartite CNTF receptor apparently occurs by the addition of the specificity-conferring element CNTFRα. Both CNTF and LIF trigger the association of initially separate receptor components, which in turn results in tyrosine phosphorylation of receptor subunits. Unlike the IL-6 receptor complex in which homodimerization of gp130 appears to be critical for signal initiation, signaling by the CNTF and LIF receptor complexes depends on the heterodimerization of gp130 with LIFRβ. Ligand-induced dimerization of signal-transducing receptor components, also seen with receptor tyrosine kinases, may provide a general mechanism for the transmission of a signal across the cell membrane.

Ciliary neurotrophic factor, named for its ability to maintain survival of ciliary neurons, is now known to have a much broader spectrum of activities; almost all of these activities, however, are restricted to cells of the nervous system (*1*). Molecular cloning of a CNTF binding protein (CNTFRα) (*2*) revealed that it was related to one of the two components of the IL-6 receptor (here designated IL-6Rα) (*3, 4*). The finding that CNTF and IL-6 bind related receptor proteins was consistent with the observation that CNTF is distantly related to a family of cytokines that includes IL-6 as well as LIF and Oncostatin M (*5*). It has recently been shown that CNTF and its distant cytokine relatives all share the use of a signal-transducing receptor component, gp130, which was initially identified as the signal transducer for IL-6 (*6–11*). A LIF binding protein (here designated LIFRβ) has been cloned (*12*) that, in combination with gp130, forms high-affinity binding sites for both LIF and Oncostatin M (*9*). Although the role of LIFRβ as a partner of gp130 appears to be similar to that of IL-6Rα and CNTFRα, its structure more closely resembles that of gp130 itself (*12*). Thus, LIFRβ might have a signal-transducing role like that of gp130.

Whereas gp130 is tyrosine-phosphorylated in response to IL-6, both gp130 and another protein the size of LIFRβ are tyrosine-phosphorylated in response to CNTF or LIF (*8*). We have therefore proposed that LIF and CNTF initiate signaling by inducing heterodimerization between gp130 and LIFRβ and, by analogy, that IL-6 acts by inducing homodimerization of gp130 (*8, 11*). We also suggested that the signal transducers gp130 and LIFRβ together could comprise a functional LIF receptor complex (*8, 9*) and that addition of the CNTFRα chain to this complex might be sufficient to convert this bipartite LIF receptor into a tripartite CNTF receptor (*8*). The limited distribution of CNTFRα (predominantly in the nervous system) apparently restricts CNTF to neuronal actions (*13*).

To determine the composition of the CNTF receptor complex, we undertook reconstitution experiments in COS cells in which various combinations of CNTFRα, gp130, or LIFRβ were expressed. The components were expressed as fusion proteins that contained epitope tags to allow for immunoprecipitation and detection. Cells were treated with either LIF or CNTF and then examined for the formation of complexes between the components, as defined by the ability of one component to co-immunoprecipitate with another in a ligand-dependent manner. Functional activation of the complex was assessed by the induction of tyrosine phosphorylation of gp130 and LIFRβ (*14*). In cells transfected with gp130 and LIFRβ, LIF triggered their association and tyrosine phosphorylation, but CNTF did not (Fig. 1). In cells transfected with all three components, CNTF triggered the association of gp130 with both LIFRβ and CNTFRα and induced tyrosine phosphorylation of gp130 and LIFRβ (Fig.

S. Davis, T. H. Aldrich, N. Stahl, L. Pan, N. Y. Ip, G. D. Yancopoulos, Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591.
T. Taga, Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan.
T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, and Department of Medicine III, Osaka University Medical School, 1-1-50, Fukushima, Fukushima-ku, Osaka 553, Japan.

*To whom correspondence should be addressed.