

The Functional Role of CD8⁺ T Helper Type 2 Cells

By Robert A. Seder* and Graham G. Le Gros†

From the *Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-1892; and †The Malaghan Institute of Medical Research, Wellington South, New Zealand

The seminal discovery by Mosmann and Coffman (1) that long-term CD4⁺ T cell clones could be segregated into subsets that produce distinct types of cytokines opened the way for extensive study of how these subsets are generated and what role they play in immune regulation as it relates to disease. Following this observation it quickly became evident in a murine model of Leishmaniasis that CD4⁺ T cells from mice that produced a Th1 response characterized by production of IFN- γ were cured, while mice expressing a Th2 response characterized by IL-4 production were susceptible to disease (2). These studies established that functional T helper subsets existed in vivo. After these observations several groups became interested in the mechanism by which naive CD4⁺ T cells differentiated into Th1 or Th2 type cells. From these studies it became clear that lymphokines themselves exert a powerful regulatory influence on this process (3, 4). Moreover, IL-4 was shown to be required for Th2 differentiation whereas IL-12 strikingly enhances the process of Th1 development. These in vitro studies formed the basis of similar types of experiments with CD8⁺ T cells. The recent reports documenting the ability of CD8 T cells to develop activities normally associated with CD4⁺ Th2 lymphocytes encourage us to suggest the mechanism whereby CD8⁺ T cells are involved in immune regulation.

Development of CD8⁺ Th2 Cells In Vitro

In an early study it was demonstrated that naive CD8⁺ T cells cultured in vitro on anti-CD3-coated dishes in the presence of IL-4, developed into CD8 T cells that produced IL-4 upon restimulation (5). These findings were extended by Erard et al. (6) using mitogens or allostimulation in the presence of IL-4 to demonstrate that the CD8 T cells not only switched to IL-4, IL-5, and IL-10 production but lost the potential for IFN- γ /IL-2 production and cytotoxic activity. Also the CD8 cells were found to be able to help B cells to antibody production through the expression of the CD40L.

The article in a recent issue of *The Journal of Experimental Medicine* by Croft et al. (7) extends this phenomenon to more physiological antigens by studying the outcome of priming for CD8⁺ T cells using cells from mice transgenic for an TCR- α/β that recognizes H-Y on Db. These workers demonstrated that by activating CD8⁺ T cells in the presence of IL-2 or IL-12 for 4 d, they could generate CD8⁺ T cells that were able to produce IL-2 and IFN- γ upon reactivation (7).

By contrast the presence of IL-4 during effector cell generation promoted the development of CD8 cells which produced IL-4 and IL-5 and stopped producing IFN- γ .

The potential consequences of a host CD8 T cell response being switched from delayed type hypersensitivity type effector functions to the so called Th2 pattern is discussed below.

The Relationship of "Th2-Like" CD8 T Cells to Disease

The IL-4-induced induction of CD8⁺ Th2-like T cells could have great relevance to immune responses against infectious agents. Specifically in situations where the cytotoxic activity and IFN- γ production by CD8 T cells are protective, the switch to IL-4, IL-5 production would probably allow a pathogen to escape elimination. One such example, may be the in vivo studies performed by Actor et al. (8) who used a helminth infection to generate a Th2-type response and then challenged the mice with a recombinant vaccinia virus. They found that the anti-viral cytotoxic activity of the CD8⁺ T cells was suppressed by the helminth-induced Th2 immune response, and that this was associated with a delayed virus clearance from the host (8). Another example of such a phenomenon could be the IL-4 and IL-5 producing CD8 T cells seen in the lesions of lepromatous leprosy patients (9).

In a separate study we investigated whether virus specific CD8 cells could switch to IL-5 production and contribute to the lung inflammation characteristic of asthma (9a). Antiviral CD8 responses were studied in transgenic mice expressing a lymphocytic choriomeningitis virus peptide specific TCR- α/β in CD8 T cells. If these transgenic mice mounted a Th2-type immune response to an unrelated antigen before receiving the virus peptide challenge, a significant airway eosinophilia was induced. In vitro analysis of the CD8 cells from the immunized mice revealed that they had switched to the production of IL-5. Taken together these results indicate that virus specific CD8 cells can switch effector functions in vivo and, interestingly, could explain the observed link between virus infection and acute exacerbation of asthma.

The Good Side of Th2 like CD8 T Cells

Using rat and murine models of experimental autoimmune encephalomyelitis (EAE) Weiner et al. (10) support the view that CD8⁺ T cells might indeed have an important role in protecting animals from EAE. They clearly show that TGF- β and possibly IL-4 production by CD8⁺ T cells, after oral feeding with antigen, prevents mice from developing EAE.

In this regard, CD8⁺ T cells that produce Th2 cytokines may provide a "suppressor" or anti-inflammatory function that is beneficial to the host.

Do Th2-like CD8 T Cells Have a Role in AIDS?

Recent studies have suggested that HIV-1-specific cytotoxic CD8⁺ T cells have a protective (although transient) role against the onset of AIDS (11). During the protective phase there is a high frequency of HIV-1-specific CD8⁺ T cells that exhibit good cytotoxic activity against HIV-1-infected cell targets. In patients whose HIV-1 infection has progressed to AIDS there is a significant reduction in the frequency of HIV-1-specific cytotoxic CD8 cells (12). In the same patients the frequency of Epstein-Barr virus (EBV)-specific cytotoxic cells is not diminished. This brings in to question the role that a Th2 response has in HIV infection and whether there is an influence of IL-4 on CD8 T cell function.

The immunoregulatory influence of IL-4 on CD8⁺ T cells in HIV infected individuals was recently addressed in the August edition of this journal by Maggi et al. (13). They generated CD8⁺ T cell clones by limiting dilution analysis in response to mitogen and IL-2 from two patients with low CD4 T cell counts and a Job's like syndrome (eczematous dermatitis, recurrent skin and sinopulmonary infections, hyper IgE). They were able to generate many clones that expressed a Th2 phenotype in response to anti-CD3 and PMA. Moreover, they found that the cytolytic activity of clones from the patient with a Job's like syndrome was significantly reduced compared to CD8⁺ Th1 clones from HIV seronegative individuals. This striking observation demonstrates that CD8 cells in a situation where high amounts of IL-4 might be present (i.e., Job's syndrome) show diminished functional cytolytic activity. One caveat to these studies is that CD8⁺ Th2 clones from HIV seronegative individuals with or without Job's syndrome or seropositive individuals not afflicted with the Job's type illness were not examined for evidence of reduced cytolytic activity. In a similar study in this issue of *The Journal of Experimental Medicine*, Paganelli et al. (14) studied a small number of HIV infected patients with CD4 counts <200, and a syndrome characterized by high IgE levels, chronic dermatitis, repeated Staphylococcal abscesses and Candida infection. They were able to demonstrate that a majority of CD8⁺ T cell lines and clones produced IL-4 in response to anti-CD3. Moreover, they showed that the IL-4 produced by the CD8⁺ T cells was able to induce IgE production from normal B cells in the presence of anti-CD40 costimulation. These authors raise the question of whether these CD8⁺ Th2-type cells, which they isolate very late in disease, appear as a consequence from an earlier Th2-dominated response. In this regard, it is clear from the studies outlined earlier that IL-4 is essential for CD8 cells to develop into IL-4 producers, then the question remains as to what is its source? Clerici et al. (15, 16) have done extensive work on characterizing the proliferative and cytokine response using peripheral blood mononuclear cells from HIV infected individuals stimulated with various antigens. They identified a group of pa-

tients whose cells are unable to proliferate or generate IL-2 in response to recall antigen but maintain these responses to alloantigens and mitogens. Moreover, in response to PHA these patients were capable of producing IL-4. They further argued that the Th2 phenotype correlates with a poor outcome. Based on this data one could speculate that in these specific patients there exists the development of a vicious circle during HIV-1 infection which depletes and exhausts the specific cytotoxic CD8 cells by their IL-4-driven conversion into CD8 Th2-like cells. Clearly, pathogens, opportunistic infections and genetic factors would greatly influence the onset of such a vicious circle by their activation of IL-4-producing CD4⁺ T cells. Lastly, the presence of IL-4 might also influence the types of T cells that develop. This is supported by the work previously noted by Erard et al. (6) showing conversion of CD8⁺ T cells into CD3⁺CD4⁻CD8⁻ when cells are activated in the presence of IL-4, and in the paper by Maggi et al. (13) which showed that several CD3⁺CD4⁻CD8⁻ clones derived from HIV-infected patients at end stage disease produced Th2 cytokines. Consistent with this speculation, previous studies of large groups of HIV-1-infected individuals have reported a significant increase in the absolute number of CD3⁺CD4⁻CD8⁻ peripheral blood lymphocytes in the late stages of AIDS (17).

One final caveat to all of these studies is that the ability to detect IL-4 protein in human disease, even from CD4⁺ T cells (the primary IL-4-producing cell) is usually in response to polyclonal stimulants such as anti-CD3, PHA, or PMA and rarely if ever detected by specific antigen. Moreover CD8⁺ Th2 clones that are generated in most of the studies cited here are rarely antigen-specific and again derived in the presence of mitogen. Whether antigen-specific CD8⁺ Th2 cells or clones can be derived directly in vivo remains a challenging task.

Conclusion

The demonstration that IL-4 can induce mature CD8⁺ T cells to develop Th2-type effector functions and lose cytotoxic activity could have a major impact upon the way in which the role of CD8 T cells in health and disease is investigated or therapeutically manipulated. It is too early to know whether these noncytotoxic, Th2-like CD8 cells are relevant as effector cells in antiviral immune responses. However, there is mounting evidence for their role in suppression of CD8⁺ T cell-mediated cytotoxicity and IFN- γ production. Alternatively, CD8⁺ Th2-type cells might have a desirable role as "suppressor" or anti-inflammatory cells through its production of "helper" cytokines. We would emphasize that the classical nomenclature of helper and suppressor cells is no longer useful in a general sense.

It is now fashionable to consider that many of the phenomena of the '70s and early '80s which were regarded as mediated by a unique population of suppressor T cells were, in fact, secondary to the differential production of cytokines by either subsets of CD4 or CD8 T cells. Although the identification of a unique subpopulation of CD8 cells with a Th2 line phenotype raises the possibility that these cells

may have been responsible for many of the manifestations of suppressor cells, it is worth noting that the majority of

the assays for suppressor cell activity involved suppression of antibody production as well as delayed type hypersensitivity.

We would like to thank Dr. Ethan Shevach for critically reviewing this manuscript and Dr. Ronald Germain for his thoughtful advice.

Address correspondence to Dr. Robert A. Seder, Lymphokine Regulation Unit, Laboratory of Clinical Investigation, NIAID, Building 10, Room 11C215, Bethesda, MD 20892.

References

1. Mosmann, T.R., H. Cherwinski, M.W. Bond., M.A. Giedlin, and R.L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities, and secreted proteins. *J. Immunol.* 136:2348-2357.
2. Heinzel, F.P., M.D. Sadick, B.J. Holaday, R.L. Coffman, and R.M. Locksley. 1989. Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *J. Exp. Med.* 169:59-72.
3. Swain, S.L., L.M. Bradley, M. Croft, S. Tonkonagy, G. Atkins, A.D. Weinberg, D.D. Duncan, S.M. Hedrick, R.W. Dutton, and G. Huston. 1991. Helper T-cell subsets: phenotype, function and the role of lymphokines in regulating their development. *Immunol. Rev.* 123:115-144.
4. Seder, R.A., and W.E. Paul. 1994. Acquisition of lymphokine producing phenotype by CD4⁺ T cells. *Annu. Rev. Immunol.* 12:635-637.
5. Seder, R.A., J.L. Boulay, F. Finkelman, S. Barbier, S.Z. Ben-Sasson, G.G. Le Gros, and W.E. Paul. 1992. CD8⁺ T cells can be primed in vitro to produce IL-4. *J. Immunol.* 148:1652-1656.
6. Erard, F., M.T. Wild, J.A. Garcia-Sanz, and G.G. Le Gros. 1993. Switch of CD8 T cells to noncytolytic CD8⁻CD4⁻ cells that make TH2 cytokines and help B cells. *Science (Wash. DC)*. 260:1802-1805.
7. Croft, M., L. Carter, S.L. Swain, and R.W. Dutton. 1994. Generation of polarized antigen-specific CD8 effector populations: reciprocal action of interleukin (IL)-4 and IL-12 in promoting type 2 versus type 1 cytokine profiles. *J. Exp. Med.* 180:1715-1728.
8. Actor, J.K., M. Shirai, M.C. Kullberg, R.M. Buller, A. Sher, and J.A. Berzofsky. 1993. Helminth infection results in decreased virus-specific CD8⁺ cytotoxic T-cell and Th1 cytokine responses as well as delayed virus clearance. *Proc. Natl. Acad. Sci. USA.* 90:948-952.
9. Salgame, P., J.S. Abrams, C. Clayberger, H. Goldstein, J. Convit, R.L. Modlin, and B.R. Bloom. 1991. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Science (Wash. DC)*. 254:279-281.
- 9a. Coyle, A.J., F. Erard, C. Bertrand, S. Walti, H. Pircher, and G. Le Gros. Virus-specific CD8⁺ cells can switch to interleukin 5 production and induce airway eosinophilia. *J. Exp. Med.* In press.
10. Weiner, H.L., A. Friedman, A. Miller, S.J. Khoury, A. Al-Sabbagh, L. Santos, M. Sayegh, R.B. Nussenblatt, D.E. Trentham, and D.A. Hafler. 1994. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu. Rev. Immunol.* 12:809-837.
11. Riddell, S.R., M.J. Gilbert, and P.D. Greenberg. 1993. CD8⁺ cytotoxic T cell therapy of cytomegalovirus and HIV infection. *Curr. Opin. Immunol.* 5:484-491.
12. Carmichael, A., X. Jin, P. Sissons, and L. Borysiewicz. 1993. Quantitative analysis of the human immunodeficiency virus type 1 (HIV-1)-specific cytotoxic T lymphocyte (CTL) response at different stages of HIV-1 infection: differential CTL responses to HIV-1 and Epstein-Barr virus in late disease. *J. Exp. Med.* 177:249-256.
13. Maggi, E., M.G. Giudizi, R. Biagiotti, F. Annunziato, R. Manetti, M.-P. Piccinni, P. Parronchi, S. Sampognaro, L. Gianmarini, G. Zuccati, et al. 1994. Th2-like CD8⁺ T cells showing B cell helper function and reduced cytolytic activity in human immunodeficiency virus type 1 infection. *J. Exp. Med.* 180:489-495.
14. Paganelli, R., E. Scala, I.J. Ansotegui, C.M. Ausiello, E. Halapi, E. Fanales-Belasio, G. D'Offizi, I. Mezzaroma, F. Pandolfi, M. Fiorilli, et al. 1994. CD8⁺ T lymphocytes provide helper activity for IgE synthesis in human immunodeficiency virus-infected patients with hyper-IgE. *J. Exp. Med.* 181:423-428.
15. Clerici, M., F.T. Hakim, D.J. Venzon, S. Blatt, C.W. Hendrix, T.A. Wynn, and G.M. Shearer. 1993. Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. *J. Clin. Invest.* 91:759-765.
16. Clerici, M., and G.M. Shearer. 1993. A TH1 → TH2 switch is a critical step in the etiology of HIV infection. *Immunol. Today.* 14:107-111.
17. Margolick, J.B., V. Carey, A. Munoz, B.F. Polk, J.V. Giorgi, K.D. Bauer, R. Kaslow, and C. Rinaldo. 1989. Development of antibodies to HIV-1 is associated with an increase in circulating CD3⁺CD4⁻CD8⁻ lymphocytes. *Clin. Immunol. Immunopathol.* 51:348-361.