Brief Definitive Report

The Role of Transforming Growth Factor β in the Generation of Suppression: An Interaction between CD8+ T and NK Cells

By J. Dixon Gray, Makoto Hirokawa, and David A. Horwitz

From the Division of Rheumatology and Immunology, Department of Medicine, University of Southern California School of Medicine, Los Angeles, California 90033

Summary

CD8+ T cells have suppressor effector functions, but the mechanisms involved in the generation of this activity are poorly understood. We report that natural killer (NK) cells have an important role in the acquisition of this function. CD8+ cells induce NK cells to produce transforming growth factor-β (TGF-β) which, in turn, stimulates CD8+ T cells to become suppressors of antibody production. Using a monocyte-dependent and -independent method to induce antibody production, we first observed that the addition of NK cells to CD8+ cells was required for optimal suppression. Next, we determined that the interaction of CD8+ T cells with NK cells resulted in a striking increase NK cell TGF-β mRNA and its production. This cytokine appeared to be involved in the induction of T suppressor cell activity since: (*a*) anti-TGF-β1 completely abrogated the suppression of immunoglobulin G synthesis; (*b*) TGF-β1 could substitute for NK cells in inducing CD8+ T cells to develop suppressor activity; and (*c*) a short exposure of T cells to TGF-β1 in the absence of B cells was sufficient for the generation of suppressor activity by CD8+ T cells. Interferon γ did not have this property. These studies provide strong evidence that in addition to its suppressive properties, TGF-β is involved in the generation of CD8+ T suppressor effector cells. Because NK cell function is decreased in many autoimmune diseases, these cells may fail to interact properly with these individuals' CD8+ cells in generating suppressors of aggressive anti-self responses.

Autoimmune diseases occur in genetically susceptible individuals after the breakdown of self-tolerance. Suppressor lymphocytes represent one mechanism that normally protects against autoimmunity (1, 2). CD8+ T cells have well-recognized suppressor functions, but in SLE, for example, CD8+ T cells enhance rather than suppress B cell function for reasons that are not fully understood (3). In addition to their cytolytic functions, NK cells also have suppressive properties (4–6). NK cell activity is decreased in autoimmune diseases such as SLE (7), Sjogren's syndrome (8), and multiple sclerosis (9). The regulatory role of NK cells is unclear since these cells have also been shown to support Ig production (10–12). Mechanisms reported to be involved in NK cell regulatory effects have included perturbation of Fcγ receptors (CD16) (6), or NK cell–derived cytokines such as TNF-α and IFN-γ (4, 12).

Another cytokine that has prominent effects on host defense is TGF-β. Mice with an inherited deficiency of this cytokine develop a multifocal inflammatory disease that results in organ failure and ultimately, in death (13). TGFβ is a multifunctional cytokine with predominantly suppressive effects on the immune system (14). Recently, TGFβ has been reported to selectively activate CD8+ T cells to proliferate (15). Herein, we report a novel interaction between NK and CD8+ T cells resulting in the generation of TGFβ, which, in turn, becomes involved in the activation of CD8+ lymphocytes to become suppressor effectors of antibody production.

Materials and Methods

Cell Isolation. PBMC were prepared from heparinized venous blood of healthy adult volunteers by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation. To obtain NK cells, PBMC was added to a nylon wool column and the eluted, nonadherent cells were immediately rosetted with AET-treated SRBC (16). The nonrosetting fraction was then stained with anti-CD3 and anti-CD74 (anti-HLA-DR) antibodies and depleted of reacting cells using immunomagnetic beads (Dynal, Great Neck, NY). This resultant population usually contained >98% CD11b+, <0.5% CD3+, and <0.5% CD20+ cells.

B cells were prepared by recovering the nylon adherent cells and immediately rosetting the recovered cells. The nonrosetting fraction was depleted of monocytes and functional NK cells by incubation with 40 mM L-leucine methyl ester (17). The resultant population was usually >92% CD20+ and <0.5% CD3+.

CD4+ and CD8+ cells were prepared from the nylon nonad-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/11/1937/06 $2.00
Volume 180 November 1994 1937–1942

herent cells were stained with antibodies to CD8, CD16, CD11b, and CD74. The same antibodies were used to obtain $CD8^+$ cells except that CD4 was substituted for CD8. Monocytes were prepared by adhering PBMC to plastic. After vigorous washing, the adherent cells were recovered by scraping using a cell scraper (GIBCO BRL, Gaithersburg, MD). Monocytes were irradiated with 3,000 rad from a 137 cesium source.

Reagents. Antibodies used were anti-CD3 (454, a gift from W. Stohl, University of Southern California; anti-CD3, purchased from Dako Corp., Carpinteria, CA), anti-CD4 (408, W. Stohl; anti-CD3, Dako), anti-CD8 (284.1.3, W. Stohl; anti-CD8, Dako), anti-CD11b (OKM1, American Type Culture Collection [ATCC], Rockville, MD), anti-CD20 (Leu 16, Becton Dickinson), anti-CD74 (L243, ATCC), anti-CD56 (Coulter Immunology, Hialeah, FL), and anti-CD2 (GT2, D. Cantrell, Imperial Cancer Research Fund, London, UK and OKT11, ATCC). Chicken anti-human TGFβ and chicken Ig were purchased from R&D Systems, Inc. (Minneapolis, MN).

Recombinant human IL-2 was kindly provided by Cetus, Corp. (Emeryville, CA) and TGF-β was purchased from R&D Systems. Recombinant IFN-γ was kindly provided by Genentech (South San Francisco, CA).

T Cell–dependent Antibody Production. Cultures were carried out in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) supplemented with 10% heat-inactivated FCS (Gemini Bioproducts Inc., Calabasas, CA), 10 g/ml gentamicin (Irvine Scientific), 2 mM L-glutamine (Flow Laboratories, McLean, VA), and 10 mM Hepes (United States Biochemical Corp., Cleveland, OH).

Where PWM was used to induce antibody synthesis, NK cells were cultured overnight at 37°C in the presence or absence of 100 U/ml rIL-2. These cells were then extensively washed before being added to culture wells. 0.5×10^5 $CD4^+$ T and B cells were added to the wells of a 96-well flat bottom microtiter plate (Flow Laboratories) along with 3×10^4 monocytes (plastic adherent cells irradiated with 3,000 rad). $CD8^+$ or NK cells were added generally at 0.5×10^5 cells per well although in some experiments graded concentrations of each were added to determine maximum regulatory effects. PWM (Sigma Chemical Co., St. Louis, MO) was added to the wells at 0.1 μg/ml. After 7 d at 37°C in 5% CO_2, cell-free supernatants from these cultures were harvested and assayed for IgG content by an ELISA, performed as previously described (5). Where anti-CD2 mAbs were used to induce antibody production, the NK cells were not treated with IL-2.

Cytokine mRNA and TGFβ Measurement. NK cells, $CD8^+$ cells, or both were added to $CD4^+$ cells, B cells, and monocytes which were then cultured in the presence of PWM. After 6 or 18 h, the cultures were stained with CD56-PE and CD8-FITC and then sorted by flow cytometry by positive selection. These cells were pelleted and processed for PCR analysis. Cytoplasmic RNA was extracted and cDNA was synthesized and amplified using appropriate primers whose sequences have been reported (18, 19). In two experiments, the NK cells were isolated after 18 h of incubation and cultured for a further 72 h. The supernatants were collected and assayed for TGFβ activity using an ELISA purchased from Genzyme Corp. (Cambridge, MA) and performed according to the manufacturer's instructions.

Results

Using two different methods to assess T cell–dependent B cell differentiation, we observed that the addition of $CD8^+$ cells to cultures containing $CD4^+$ and B cells generally resulted in only modest inhibition of antibody synthesis. These studies suggested that an additional mononuclear cell population was needed for the generation of potent suppressor activity. We considered that NK cells might have this property. We first tested this hypothesis using PWM to induce antibody synthesis. Neither the addition of $CD8^+$ cells, NK cells, nor both together markedly suppressed antibody synthesis. Whereas the addition of IL-2–activated NK cells did not have this effect, the combination of activated NK and $CD8^+$ cells markedly suppressed Ig production in eight separate experiments. Three experiments illustrating the range of effects observed are shown in Fig. 1. The suppressive effect of adding both NK and $CD8^+$ cells was not due to alterations in the number of cells in the wells since increased numbers

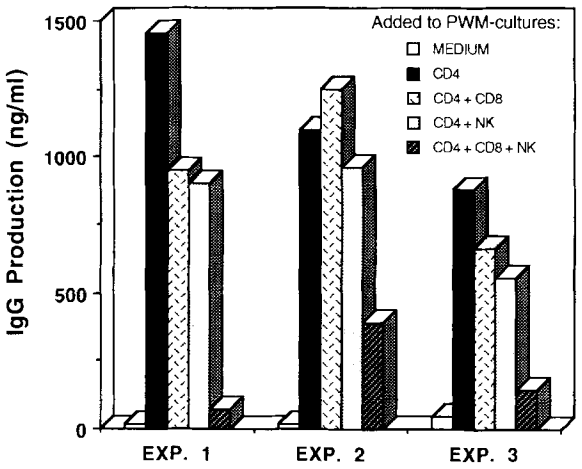

Figure 1. Marked suppression of antibody production requires both $CD8^+$ and NK cells. To B cells and irradiated monocytes (10^4) cultured with PWM were added $CD4^+$ cells, (*solid bars*), $CD4^+$ and $CD8^+$ cells (*light hatched bars*), $CD4^+$ and NK cells (*stippled bars*), and $CD4^+$, $CD8^+$, and NK cells (*dark hatched bars*). The NK cells had been pretreated with rIL-2 (100 U/ml). All lymphocyte subsets were added at 5×10^4 cells/well. After 7 d of culture with PWM, supernatants were harvested from the wells and assayed for IgG content (ng/ml) by an ELISA. (*Open bars*) Background IgG in cultures.

Table 1. *Suppression of IgM and IgG Requires Both $CD8^+$ and NK Cells*

Added to PWM cultures	Exp. 1		Exp. 2	
	IgM	IgG	IgM	IgG
Medium	4,100	875	2,800	440
CD8	3,000	650	2,400	460
$NK_{IL\text{-}2}$	3,900	550	5,200	460
$CD8 + NK_{IL\text{-}2}$	260	145	840	280

PWM cultures consisted of $CD4^+$ cells, B cells, irradiated (3,000 rad) monocytes, and PWM at the concentrations indicated in Fig. 1. To the cultures were added medium, $CD8^+$ cells, and/or NK cells at 5×10^4/well each. After 7 d of culture, supernatants were harvested and assayed for IgM and IgG content by an ELISA.

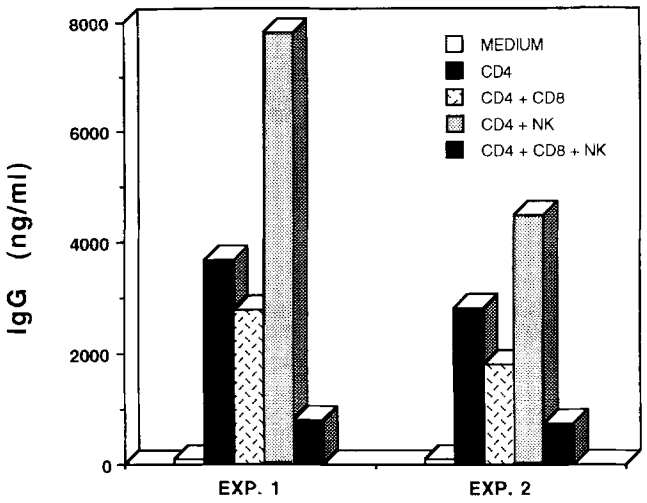

Figure 2. Marked suppression of antibody production in a monocyte-independent system requires both $CD8^+$ and NK cells. $CD4^+$ (5×10^4) and B cells (5×10^4) were cultured in the presence of a mitogenic combination of anti-CD2 antibodies (OKT11 and GT2). Added to the wells were $CD8^+$ cells (5×10^4) and/or untreated NK cells (5×10^4). After 7 d of culture, supernatants were assayed for IgG content by an ELISA.

of $CD8^+$ or NK cells alone were ineffective. In addition to suppression of IgG, IgM production was also markedly suppressed as shown in Table 1.

We have recently reported that one can assess the direct effects of T cells on B cells in the absence of exogenous accessory cells with a mitogenic combination of anti-CD2 mAbs (20). As shown in Fig. 2, with this second method we also observed that the addition of $CD8^+$ T cells to $CD4^+$ and B cells had modest suppressive effects. The addition of NK cells generally enhanced antibody production. Since NK cells display CD2 receptors, these cells were not pretreated with IL-2. Nonetheless, the presence of both $CD8^+$ and NK cells resulted in optimal suppression of IgG synthesis.

As many lymphocyte functions can be mediated by cytokines, the expression of cytokine mRNA was measured to identify possible candidates for the suppressive effect. Cytokine mRNA expression by $CD8^+$ and NK cells was determined after these cells were cultured separately or together in PWM-stimulated cultures. After 6 h of incubation, $CD8^+$ T and $CD56^+$ NK cells were sorted by positive selection. The isolated cell populations were then analyzed for the expression of cytokine mRNA by PCR. Cytokines produced by NK and $CD8^+$ cells include TNF-α, TNF-β and IFN-γ (21). Because of the inhibitory effects of TGFβ (22, 23), this cytokine was also examined.

There was only one consistent result of culturing both NK and $CD8^+$ cells together. This was a marked increase in NK cell TGFβ mRNA. In some experiments, there was also a modest increase in $CD8^+$ cell TGFβ mRNA and an increase in NK cell IFN-γ transcripts. Two experiments are shown in Fig. 3.

Using a similar protocol, two additional experiments were performed where NK cells were isolated after an overnight

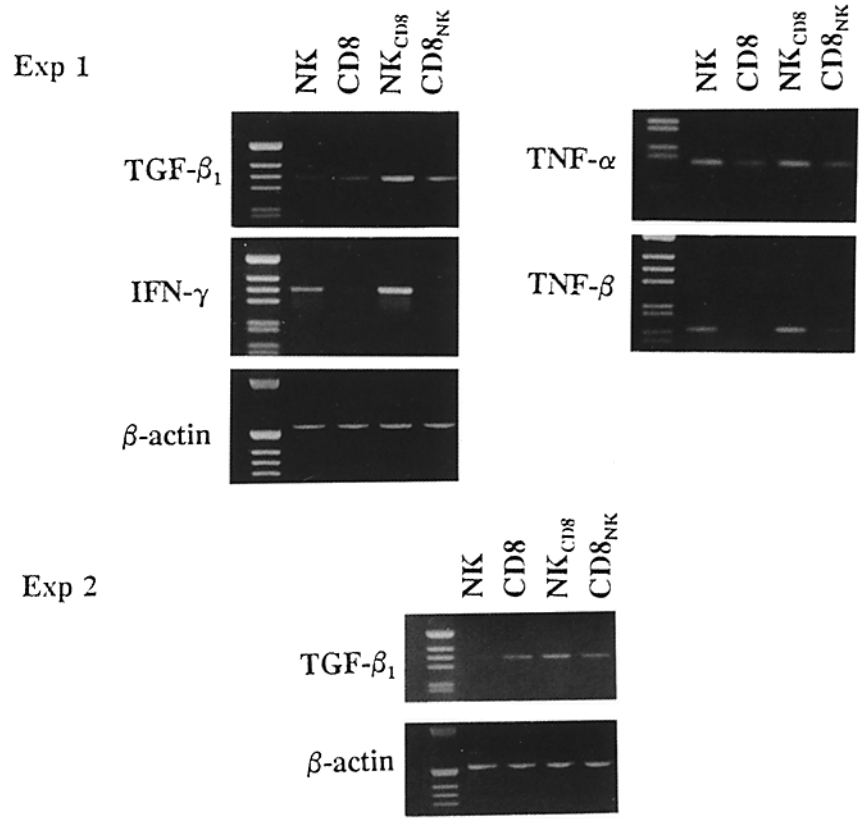

Figure 3. Cytokine mRNA expression in NK and/or $CD8^+$ cells after these cells were cultured separately or together. PWM cultures containing $CD4^+$ cells (10^6), B cells (10^6), and irradiated (3,000 rad) monocytes were cultured with: lane *1*, IL-2–pretreated NK cells (10^6); lane *2*, $CD8^+$ cells (10^6); lane *3*, NK cells after culture with CD8 cells; and lane *4*, $CD8^+$ cells after culture with NK cells. After 6 h at 37°C in 3-ml culture tubes, the cells were stained with anti-CD56-PE and anti-CD8-FITC and sorted by flow cytometry for NK and CD8 cells. Cells were then processed for PCR analysis. A similar pattern of cytokine expression was observed after 18 h of culture.

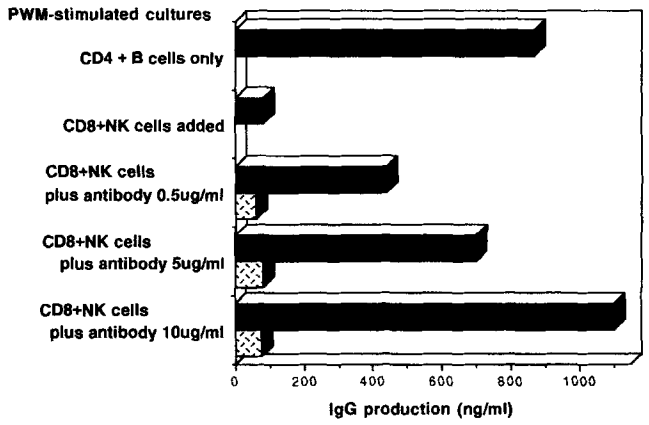

Figure 4. Anti-TGFβ abolished NK–CD8 cell-induced suppression. Cultures were initiated as described in the legend to Fig. 1. Added to these cultures were different concentrations of anti-TGFβ antibody (0.5–10 μg/ml) or similar concentrations of control chicken antibody.

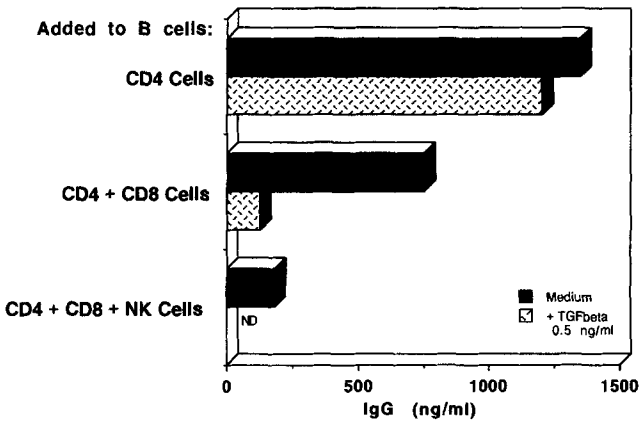

Figure 5. TGFβ can substitute for NK cells in the generation of T cell suppressor activity. TGFβ (0.5 ng/ml) or medium was added to PWM cultures containing $CD4^+$ cells, B cells, and irradiated monocytes with or without $CD8^+$ cells as described in the legend to Fig. 1. Some PWM cultures received both $CD8^+$ and NK cells that had been incubated with 100 U/ml rIL-2. After 7 d of culture, supernatants were assayed for Ig content by an ELISA.

incubation and cultured for an additional 72 h. When culture supernatants were assayed for TGFβ by an ELISA, NK cells isolated from cultures containing $CD8^+$ cells produced 660 pg/ml, whereas in the absence of $CD8^+$ cells TGFβ was <50 pg/ml.

These results raised the possibility that production of TGFβ might be important in the generation of suppressor activity. To test this hypothesis, the effect of anti-TGFβ on IgG production was determined. Graded doses of anti-TGFβ antibody were added to PWM-stimulated cultures containing both IL-2–treated NK and $CD8^+$ cells. As shown in Fig. 4, anti-TGFβ, but not the control antibody reversed the suppressive effects. These results demonstrate that TGFβ contributes to the suppression of IgG secretion.

Since NK cells can act as a source of TGFβ, we determined whether TGFβ could take the place of NK cells in the generation of suppressive activity by $CD8^+$ cells. Because of the known suppressive effects of this cytokine on antibody production, we first added graded doses of TGFβ to $CD4^+$ and B cells to determine the minimum concentration that would not be directly inhibitory. This concentration of TGFβ (0.5 ng/ml) was then added to cultures where $CD8^+$ cells were present or absent. Cultures containing both $CD8^+$ and NK cells served as the positive control. In each of three separate experiments, we have observed that the effects of adding either TGFβ or NK cells to $CD8^+$ cells are similar. In each case, there was marked suppression of IgG production. Fig. 5 shows one representative experiment.

These observations suggested that TGFβ was involved in the activation of $CD8^+$ T cells to become suppressors of antibody production. However, other explanations included the priming of B cells by TGFβ to become susceptible to the action of suppressor cells. To address this, two-stage experiments were performed where T cells were stimulated with PWM overnight in the presence or absence of TGFβ. The cells were then extensively washed before being added to B cells. Exposure of either $CD4^+$ or $CD8^+$ cells alone to TGFβ did not induce any suppressive activity. However, this activity did develop when cultures containing both $CD4^+$ and $CD8^+$ cells were preincubated with TGFβ. Both IgG and IgM production were significantly inhibited (Fig. 6).

IFN-γ is another cytokine reported to be associated with the development of suppressor activity (24). Therefore, a direct comparison was made between TGFβ and IFN-γ for their ability to induce suppressor activity using the two-step culture system. As before, preincubation of cultures containing

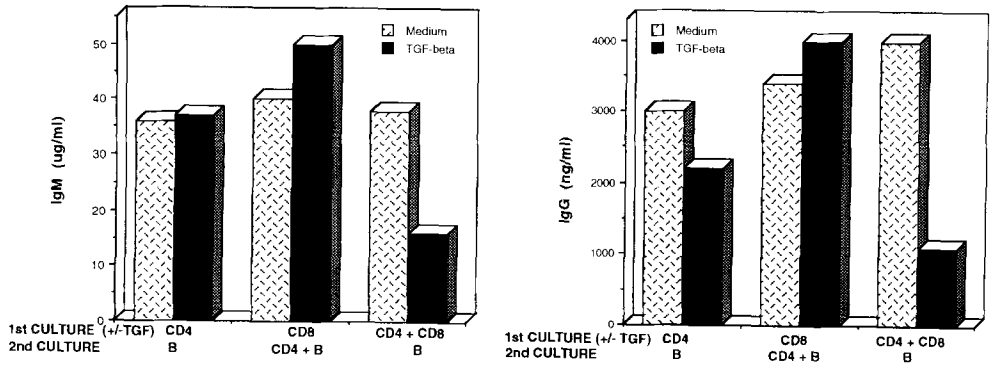

Figure 6. T lymphocytes preincubated with TGFβ mediate suppression. $CD4^+$ cells, $CD8^+$ cells, or both were cultured overnight with irradiated monocytes and PWM in the presence or absence of TGFβ (0.1 ng/ml). Next day, the wells were extensively washed. B cells and fresh PWM were then added to all the wells. In the case of the $CD8^+$ only wells, $CD4^+$ cells were also added in the second culture. All cell concentrations were as described the legend to Fig. 1.

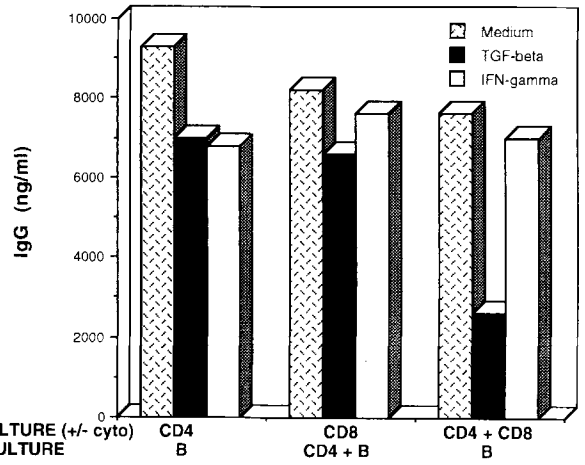

Figure 7. Comparison of TGFβ and IFN-γ on the development of suppressive activity was as described in Fig. 6, except that IFN-γ (100 U/ml) was also tested.

both $CD4^+$ and $CD8^+$ cells with TGFβ stimulates suppressor activity. However, IFN-γ, over a wide range of concentrations, did not have any suppressive effect (Fig. 7).

Discussion

In this study, $CD4^+$ cells were unable to induce $CD8^+$ cells to become potent suppressors of antibody production. It has been well established that $CD4^+$ $CD45RA^+$ cells have important roles in the development of $CD8^+$ T cell suppressor activity (25). Here we show that NK cells were also required for the development of optimal suppressor activity. This finding was observed using both a monocyte-dependent and -independent culture system to induce polyclonal B cell activation.

Previously, mechanisms responsible for the suppression of antibody production by NK cells were poorly understood. The addition of NK cells to PBMC could either decrease $CD4^+$ cell helper activity or increase $CD8^+$ cell suppressor activity. On the basis of the present experiments, the former possibility is unlikely since the addition of NK cells to $CD4^+$ and B cells did not notably decrease antibody production. In fact, when antibody production was stimulated with anti-CD2, NK cells enhanced the amount of antibody produced. What we have demonstrated is that the generation of suppression requires both NK and $CD8^+$ cells. When PWM was used to induce antibody production, the NK cells had to be preactivated with IL-2 in order for suppression to occur. When anti-CD2 was used, however, this was not the case because NK cells express CD2 on their cell surface and have been reported to be activated by these mAbs.

To identify potentially critical cytokines that might be involved in the activation of suppression, we examined NK and $CD8^+$ cells for cytokine mRNA after they were cultured together or separately in PWM-stimulated cultures. The striking observation was the upregulation of TGFβ mRNA in NK cells that had been cultured together with $CD8^+$ cells. NK cells isolated from cultures containing $CD8^+$ cells produced TGFβ, whereas the absence of $CD8^+$ cells, little or none was produced. Although TGFβ message has been described previously in activated NK cells (26), this, to our knowledge, is the first report of this cytokine produced by these cells. Moreover, the TGFβ detected in the supernatant was in the active form, indicating that conversion from the latent form had occurred.

Evidence that TGFβ was actually involved in the generation of suppressor cell activity was provided by (*a*) the observation that anti-TGFβ abolished the suppressive effects of the NK–$CD8^+$ cell interaction; and (*b*) the ability of TGFβ to replace NK cells in generating optimal $CD8^+$ cell suppressor cell activity. It is important to emphasize that with the low concentration of TGFβ used, there was no inhibitory effect of this cytokine on IgG production in the absence of $CD8^+$ cells.

TGFβ is an immunosuppressive cytokine with marked inhibitory effects on T, B, and NK cell effector functions (14). It is interesting to note that although TGFβ had a marked effect on antibody production, proliferative activity in PWM cultures was unaffected by TGFβ (data not shown). This suggests that some cellular functions are more susceptible to the effects of TGFβ than others. In addition to downregulating effector functions, the present study suggests that this cytokine can also activate lymphocytes to develop suppressive effects. This suggestion is consistent with the recent demonstration that TGFβ selectively stimulates $CD45RB_{hi}$ $CD8^+$ cells to proliferate (15). Moreover, in chickens, there is one report that TGFβ is important in the generation of $CD8^+$ cell suppressor activity (27). Other positive effects on the growth and development of lymphocyte subsets have been reported (28, 29). Our results demonstrate that TGFβ alone is insufficient to stimulate $CD8^+$ cells to become suppressor effector cells but that $CD4^+$ cells are also required. This is wholly consistent with previous reports describing the role of $CD4^+$ cell suppressor inducer cells. Our data indicate that for optimal suppression of Ig production, $CD4^+$ cells and a source of TGFβ are needed.

As stated above, NK cell function is decreased in many autoimmune diseases including SLE, Sjogren's syndrome, and multiple sclerosis (7–9). If NK cells interact with $CD8^+$ cells in a manner similar in vivo as they do in this in vitro model, then impaired NK cell function may reflect a defect that contributes to the inability of $CD8^+$ cells to effectively suppress the aggressive anti-self responses in these diseases.

The authors gratefully appreciate the technical expertise of Ms. Lillie Hsu and Hua Wang. We also thank Drs. William Stohl, Chaim Jacob and Gunther Dennert for their helpful comments.

This work was supported by grants from the National Institutes of Health (AR-29846), the Nora Eccles Treadwell Foundation, and the Southern California Chapter of the Arthritis Foundation.

Address correspondence to Dr. D. A. Horwitz, University of Southern California School of Medicine, Division of Rheumatology and Immunology, 2001 Zonal Avenue, HMR-711, Los Angeles, CA 90033. Makoto Hirokawa's present address is Third Department of Internal Medicine, Akita University School of Medicine, 1-1-1 Hondo, Akita 010, Japan.

Received for publication 25 October 1993 and in revised form 13 June 1994.

References

1. Kumar, V., and E.E. Sercarz. 1991. Regulation of autoimmunity. *Curr. Opin. Immunol.* 3:888.
2. Bloom, B.R., P. Salgame, and B. Diamond. 1992. Revisiting and revising suppressor T cells. *Immunol. Today.* 13:131.
3. Linker-Israeli, M., F.P. Quismorio, Jr., and D.A. Horwitz. 1990. $CD8^+$ lymphocytes from patients with systemic lupus erythematosus sustain rather than suppress spontaneous polyclonal IgG production and synergize with $CD4^+$ cells to support autoantibody synthesis. *Arthritis Rheum.* 33:1216.
4. Kuwano, K., S. Arai, T. Munakata, Y. Tomita, Y. Yoshitake, and K. Kumagai. 1986. Suppressive effect of human natural killer cells on Epstein-Barr virus–induced immunoglobulin synthesis. *J. Immunol.* 137:1462.
5. Abo, W., J.D. Gray, A.C. Bakke, and D.A. Horwitz. 1987. Studies on human blood lymphocytes with iC3b (type 3) complement receptors. II. Characterization of subsets which regulate pokeweed mitogen–induced lymphocyte proliferation and immunoglobulin synthesis. *Clin. Exp. Immunol.* 67:544.
6. Tilden, A.B., T. Abo, and C.M. Balch. 1983. Suppressor cell function of human granular lymphocytes identified by the HNK-1 (Leu 7) monoclonal antibody. *J. Immunol.* 130:1171.
7. Katz, P., A. Zaytoun, and J.H. Lee. 1982. Abnormal natural killer cell activity in SLE: an intrinsic defect in the lytic event. *J. Immunol.* 129:1966.
8. Goto, J., K. Tanimoto, T. Chihara, and Y. Horiuchi. 1981. Natural cell–mediated cytotoxicity in Sjogren's syndrome and rheumatoid arthritis. *Arthritis Rheum.* 24:1377.
9. Benczur, M., G.G. Petranyi, G. Palffy, M. Varga, M. Talas, B. Kotsy, I. Foldes, and S.R. Hollan. 1980. Dysfunction of natural killer cells in multiple sclerosis: a possible pathogenetic factor. *Clin. Exp. Immunol.* 39:657.
10. Vyakarnam, A., M.K. Brenner, J.E. Reittie, C.H. Houlker, and P.J. Lachmann. 1985. Human clones with natural killer function can activate B cells and secrete B cell differentiation factors. *Eur. J. Immunol.* 15:606.
11. Kimata, H., F. Shanahan, M. Brogan, S. Targan, and A. Saxon. 1987. Modulation of ongoing human immunoglobulin synthesis by natural killer cells. *Cell. Immunol.* 107:74.
12. Becker, J.C., W. Kolanus, C. Lonnemann, and R.E. Schmidt. 1990. Human natural killer clones enhance in vitro antibody production by tumour necrosis factor alpha and gamma interferon. *Scand. J. Immunol.* 32:153.
13. Shull, M.M., I. Ormsby, A.B. Kier, S. Pawlowski, R.J. Diebold, M. Yin, R. Allen, C. Sidman, G. Proetzel, D. Calvin, N. Annunziata, and T. Doetschman. 1992. Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature (Lond.).* 359:693.
14. Massague, J. 1990. The transforming growth factor-β family. *Annu. Rev. Cell Biol.* 6:597.
15. Lee, H.-M., and S. Rich. 1993. Differential activation of $CD8^+$ T cells by transforming growth factor-β1. *J. Immunol.* 151:668.
16. Abrams, S.I., and Z. Brahmi. 1988. Mechanism of K562-induced human natural killer cell inactivation using highly enriched effector cells isolated via a new single-step sheep erythrocyte rosette assay. *Ann. Inst. Pasteur Immunol.* 139:361.
17. Shau, H., and S.H. Golub. 1985. Depletion of NK cells with the lysosomotropic agent I-leucine methyl ester and the in vitro generation of NK activity from NK precursor cells. *J. Immunol.* 134:1136.
18. 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.
19. Yamamura, M., K. Uyemura, R.J. Deans, K. Weinberg, T.H. Rea, B.R. Bloom, and R.L. Modlin. 1991. Defining protective responses to pathogens with cytokine profiles in leprosy lesions. *Science (Wash. DC).* 254:277.
20. Hirokawa, M., J.D. Gray, T. Takahashi, and D.A. Horwitz. 1992. Human resting B lymphocyte can serve as accessory cells for anti-CD2–induced T cell activation. *J. Immunol.* 149:1859.
21. Cuturi, M.C., M. Murphy, M.P. Costa-Giomi, R. Weinmann, B. Perussia, and G. Trinchieri. 1987. Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. *J. Exp. Med.* 165:1581.
22. Kehrl, J.H., A.B. Roberts, L.M. Wakefield, S. Jakowlew, M.B. Sporn, and A.S. Fauci. 1986. Transforming growth factor β is an important immunomodulatory protein for human B lymphocytes. *J. Immunol.* 137:3855.
23. Kehrl, J.H., A.S. Taylor, G.A. Delsing, A.B. Roberts, M.B. Sporn, and A.S. Fauci. 1989. Further studies of the role of transforming growth factor-β in human B cell function. *J. Immunol.* 143:1868.
24. Elmasry, M.N., I.J. Fox, and R.R. Rich. 1987. Sequential effects of prostaglandins and interferon-gamma on the differentiation of $CD8^+$ suppressor cells. *J. Immunol.* 139:688.
25. Morimoto, C., N.L. Letvin, J.A. Distaso, W.R. Aldrich, and S.F. Schlossman. 1985. The isolation and characterization of the human suppressor inducer T cell subset. *J. Immunol.* 134:1508.
26. Vitolo, D., N.L. Vujanovic, H. Raboniwich, M. Schlesinger, R.B. Herberman, and T.L. Whiteside. 1993. Rapid IL-2–induced adherence of human natural killer cells. Expression of mRNA for cytokines and IL-2 receptors in adherent NK cells. *J. Immunol.* 151:1926.
27. Quere, P., and G.J. Thorbecke. 1990. Multiple suppressive effects of transforming growth factor β1 on the immune response in chickens. *Cell. Immunol.* 129:468–477.
28. Swain, S.L., G. Huston, S. Tonkonogy, and A. Weinberg. 1991. Transforming growth factor-β and IL-4 cause helper T cell precursors to develop into distinct effector helper cells that differ in lymphokine secretion pattern and cell surface phenotype. *J. Immunol.* 147:2991.
29. Coffman, R.L., D.A. Lebman, and B. Shrader. 1989. Transforming growth factor β specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. *J. Exp. Med.* 170:1039.