Enhancement of In Vitro and In Vivo Antigen-specific Antibody Responses by Interleukin 11

By Tinggui Yin,* Paul Schendel,‖ and Yu-Chung Yang*‡§

*From the *Division of Hematology/Oncology, Department of Medicine, and ‡Department of Biochemistry/Molecular Biology; §Walther Oncology Center, Indiana University School of Medicine, Indianapolis, Indiana 46202; and ‖Genetics Institute, Cambridge, Massachusetts 02140*

Summary

The availability of large quantities of highly purified recombinant interleukin 11 (rhuIL-11) has allowed us to investigate the effects of rhuIL-11 on sheep red blood cell (SRBC)-specific antibody responses in the murine system. The results showed that rhuIL-11 was effective in enhancing the generation of mouse spleen SRBC-specific plaque-forming cells (PFC) in the in vitro cell culture system in a dose-dependent manner. These effects of rhuIL-11 were abrogated completely by the addition of anti-rhuIL-11 antibody, but not by the addition of preimmunized rabbit serum. Cell-depletion studies revealed that L3T4 $(CD4)^+$ T cells, but not Lyt-2 $(CD8)^+$ T cells, are required in the rhuIL-11-stimulated augmentation of SRBC-specific antibody responses. The effects of rhuIL-11 on the SRBC-specific antibody responses in vivo were also examined. RhuIL-11 administration to normal C3H/HeJ mice resulted in a dose-dependent increase in the number of spleen SRBC-specific PFC as well as serum SRBC-specific antibody titer in both the primary and secondary immune responses. In mice immunosuppressed by cyclophosphamide treatment, rhuIL-11 administration significantly augmented the number of spleen SRBC-specific PFC as well as serum SRBC-specific antibody titer when compared with the cyclophosphamide-treated mice without IL-11 treatment. These results demonstrated that IL-11 is a novel cytokine involved in modulating antigen-specific antibody responses in vitro as well as in vivo.

Growth and differentiation of B lymphocytes are regulated at least in part by many soluble factors called cytokines that are produced by a variety of cells (1–3). Although the exact number of cytokines and the precise mechanisms of cytokine action involved in this process are still under intensive investigation, a number of cytokines such as IL-2 (4–6), IL-4 (7), IL-5 (8, 9), IL-6 (5, 6, 10, 11), IFN-γ (7, 12), and IL-10 (13) have been implicated to play an important role in modulating B cell activation, proliferation, and/or differentiation.

Recently, a new cytokine designated as IL-11 was identified in medium conditioned by a cell line derived from primate bone marrow stromal cells (14). The cDNA encoding primate IL-11 was cloned by expression cloning method based on the ability of this cytokine to stimulate the proliferation of an IL-6–dependent mouse plasmacytoma cell line, T1165 (14). The human homologue of primate IL-11 was subsequently cloned and expressed in mammalian cells (14). Initial studies have shown that IL-11 is able to increase the number of antibody-forming cells in murine spleen cell cultures and to augment IL-3–dependent development of megakaryocyte colonies in both human and mouse bone marrow cultures. Recent studies have also shown that recombinant human IL-11 (rhuIL-11), like IL-6, can bring the pluripotent stem cells out of the G_0 stage of the cell cycle to respond to intermediate or late-acting growth factors such as IL-3 or granulocyte/macrophage CSF (15). These results imply that rhuIL-11[1] is likely to be a multifunctional mediator in the complicated cytokine network.

Since many biological activities of IL-11 overlapped with those of IL-6, and IL-6 has been shown to play an important role in modulating immune responses, we speculated that IL-11 may also contribute to the immunologic events critical to the host defense system. As a first step of understanding the role of IL-11 in regulating immune responses, we tested the effects of rhuIL-11 on antigen (Ag)-specific antibody responses in vitro and in vivo. The results demonstrated that IL-11 significantly enhanced in vitro Ag-specific antibody responses and augmented Ag-specific antibody responses in both normal mice and mice immunosuppressed by cyclophosphamide (CYC) treatment.

[1] *Abbreviations used in this paper:* Ag, antigen; CYC, cyclophosphamide; PFC, plaque-forming cells; rhuIL-11, recombinant human interleukin 11.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/01/0211/06 $2.00
Volume 175 January 1992 211–216

Materials and Methods

Mice. C57BL/6J and C3H/HeJ female mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice aged 8–12 wk were used in all the experiments.

RhuIL-11. RhuIL-11 produced in *Escherichia coli* and purified to homogeneity was obtained from Genetic Institute (Cambridge, MA). Purified rhuIL-11 had a specific activity of 1.8×10^6 U/mg by T1165 cell proliferation assay (14) and contained <1.25 ng endotoxin/mg protein.

Antibodies. Anti-Thy-1.2 mAb, anti-L3T4 mAb, and anti-Lyt-2 mAb were purchased from Cedarlane Lab (Ontario, Canada). Polyclonal rabbit anti-rhuIL-11 antiserum was a generous gift from Dr. Ed Alderman (Genetics Institute).

Cell Preparation. To deplete $CD3^+$, $CD4^+$, or $CD8^+$ cells, murine spleen cells were incubated with anti-Thy-1.2 mAb, anti-L3T4 mAb, or anti-Lyt-2 mAb in RPMI 1640 supplemented with 0.5% BSA, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 5×10^{-5} M β-mercaptoethanol (hereafter referred to as treated medium). After a 1-h incubation at 4°C, cells were washed three times in treated medium and resuspended in treated medium containing 1:15 dilution of rabbit complement (Cedarlane Lab). After incubation at 37°C for 40 min with complement, cells were washed three times in treated medium and resuspended in IMDM (Sigma Chemical Co., St. Louis, MO) supplemented with 10% FCS (Hyclone, Logan, UT), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 5×10^{-5} M β-mercaptoethanol (hereafter referred to as complete culture medium).

Assay for SRBC-Specific PFC In Vitro. Untreated or treated spleen cells (2×10^6) were cultured in 24-well flat-bottomed plates (Becton Dickinson & Co., Lincoln Park, NJ) in a volume of 0.5 ml of complete culture medium with 4×10^5 to 10^6 SRBC in the presence or absence of various concentrations of rhuIL-11. The plates were placed on a rocker platform at 7–10 complete cycles per min (16) and incubated for 4 or 5 d at 37°C in a humidified atmosphere of 7% O_2, 10% CO_2, and 83% N_2. At the end of culture period, cells were collected, washed once with RPMI 1640 (Sigma Chemical Co.), and resuspended in RPMI 1640 supplemented with 2 mM L-glutamine. The number of SRBC-specific plaque-forming cells (PFC) per 10^6 spleen cells was identified with petri-dish plaque assay as described by Dresser (17).

Determination of SRBC-Specific PFC and Antibody Titer In Vivo. SRBC were washed three times, resuspended in pyrogen-free PBS, and inoculated intraperitoneally at 2×10^6 (for secondary response) or 4×10^7 (for primary response)/0.2 ml/mouse. On the same day of antigen inoculation, groups of three mice received injections twice daily (once intraperitoneally, once subcutaneously) with 0.2 ml/mouse of 2.5% syngeneic mouse serum in PBS containing various concentrations of rhuIL-11 for 6 d. Control mice received the same volume of solution without IL-11. At 7 d, mice were killed, spleen cells and serum collected, and SRBC-specific PFC were assayed as described above. Anti-SRBC-specific antibody titer in serum was measured by a hemagglutination test (6). Briefly, 50 μl of a twofold serial dilution of mouse immune serum was mixed with 50 μl of 1% SRBC suspension containing 1% BSA in PBS. The agglutination titer was determined after a 6-h incubation at 4°C as the reciprocal of the highest dilution giving distinct agglutination.

CYC Treatment. CYC (Sigma) was dissolved in pyrogen-free saline and injected intraperitoneally at a dose of 200 mg/kg body weight. 4 h after CYC treatment, each mouse received 4×10^7 SRBC intraperitoneally. RhuIL-11 (1-μg/injection, once intraperitoneally, once subcutaneously) or control solution treatment began the next day and continued for the duration of the experiment. At various times, groups of three mice were killed and splenic PFC as well as serum antibody titer were determined as described above.

Measurement of Total Ig Concentration in Serum. The total amount of Ig in mouse serum was determined by ELISA as described by Luzuy et al. (18).

Statistical Analysis. Statistical significance was assessed by the Student's *t* test. Probabilities were calculated by two-tailed analysis.

Results and Discussion

Effect of rhuIL-11 on the Primary Immune Response to SRBC. We examined the effect of purified rhuIL-11 on the primary immune response to SRBC of spleen cells from C57BL/6J mice. Mouse spleen cells were cultured with SRBC in the presence of various concentrations of rhuIL-11. Specific direct PFC were counted on day 5, previously determined to be the optimal period for the PFC responses (our unpublished results). As shown in Fig. 1, rhuIL-11 induced IgM PFC response in a dose-dependent manner. The addition of as low as 1 U/ml rhuIL-11 could increase IgM PFC 3.3-fold. At the concentration of 500 U/ml, rhuIL-11 increased IgM PFC 6.7-fold. These results suggested that rhuIL-11, like IL-6 (6), is a strong inducer of SRBC-specific PFC in vitro. In addition, the kinetic studies showed that the addition of rhuIL-11 48 h after the initiation of spleen cell culture failed to increase Ag-specific PFC (data not shown), suggesting that rhuIL-11, unlike IL-6 (6), has to be added at an early stage of cell culture in order to elevate Ag-specific PFC.

Effect of rhuIL-11 on the Immune Response to SRBC Using Spleen Cells from SRBC-Primed Mice. As rhuIL-11 is an effective enhancer of SRBC-specific IgM PFC in the primary immune response, we next examined whether rhuIL-11 augments the in vitro response to spleen cell primed with SRBC Ag in vivo. Mice were immunized intraperitoneally with 4×10^7 SRBC/mouse. 2 d after immunization, spleen cells were removed and restimulated in vitro with SRBC in the presence of rhuIL-11. On day 4 (previously determined to be the

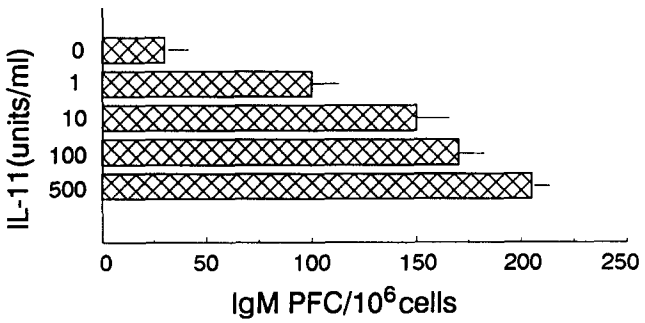

Figure 1. Effect of rhuIL-11 on primary immune response to SRBC in vitro. C57BL/6J mouse spleen cells (2×10^6/well in 0.5 ml medium) were cultured with 10^6 SRBC in the presence of various concentrations of rhuIL-11 in 24-well plates as described in Materials and Methods. SRBC-specific direct PFC were counted on day 5 of the culture. The data are the arithmetic means (± SD) of duplicate cultures. Three additional experiments showed similar results. The spleen cells cultured with IL-11 in absence of SRBC did not show any SRBC-specific PFC.

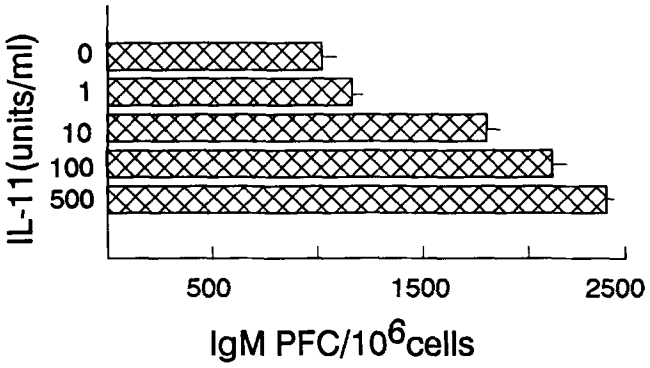

Figure 2. Effect of rhuIL-11 on in vivo SRBC-primed spleen cell response to SRBC in vitro. C57BL/6J mice were injected with 4 × 10^7 SRBC/mouse i.p. 2 d after immunization, spleen cells (2 × 10^6/well in 0.5 ml medium) were restimulated with 4 × 10^5 SRBC in the presence of various concentrations of rhuIL-11. SRBC-specific direct PFC were counted on day 4 of the culture. The data are the arithmetic mean (±SD) of duplicate cultures. Three additional experiments showed similar results.

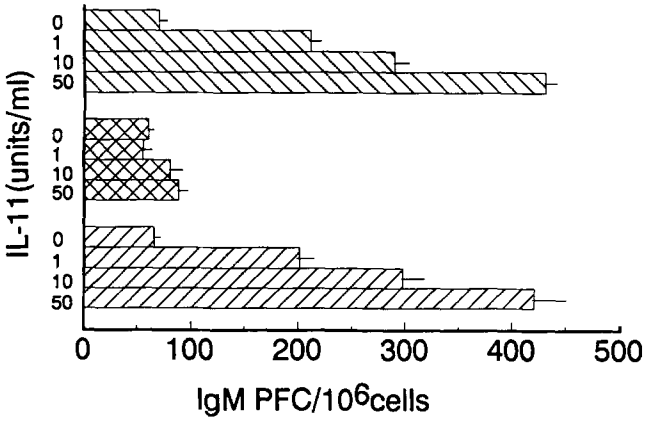

Figure 3. Neutralization of rhuIL-11-induced enhancement of PFC in primary immune response to SRBC by anti-rhuIL-11 antibody. C57BL/6J mouse spleen cells (2 × 10^6/well in 0.5 ml medium) were cultured with 10^6 SRBC in the presence of indicated amounts of rhuIL-11 (▨) or rhuIL-11 neutralized by incubation with 50 neutralization units of anti-rhuIL-11 antibody (▨) or preimmunized rabbit serum (▨) at 37°C for 2 h. SRBC-specific direct PFC were counted on day 5 of the culture. The data are expressed as the mean (±SD) of duplicate cultures. Representative results of two separate experiments are shown.

optimal period for the PFC response), SRBC-specific PFC were assayed. It was demonstrated that rhuIL-11 augmented IgM PFC in a dose-dependent manner when spleen cells from SRBC-immunized mice were used (Fig. 2). However, SRBC-primed spleen cells cultured in vitro without SRBC restimulation failed to respond to rhuIL-11 in eliciting the SRBC-specific PFC response (data not shown). These results indicated that rhuIL-11 could not induce the differentiation of antigen-primed B cells to become antibody-producing cells without antigen restimulation. The kinetic studies using in vivo primed spleen cell culture system showed that rhuIL-11, unlike IL-6 (6), has to be added at an early stage of cell culture in order to significantly augment Ag-specific PFC.

Abrogation of rhuIL-11–induced Augmentation of SRBC-specific PFC Responses by anti–rhuIL-11 Antibody. To demonstrate rhuIL-11 as an enhancer of SRBC-specific PFC, the effect of anti-rhuIL-11 antibody on the rhuIL-11–induced augmentation of SRBC-specific PFC was examined. Addition of 50 neutralization units of anti–rhuIL-11 antibody totally abolished the capacity of rhuIL-11 to increase the production of SRBC-specific PFC (Fig. 3), whereas the addition of same concentration of preimmunized rabbit serum had no effect

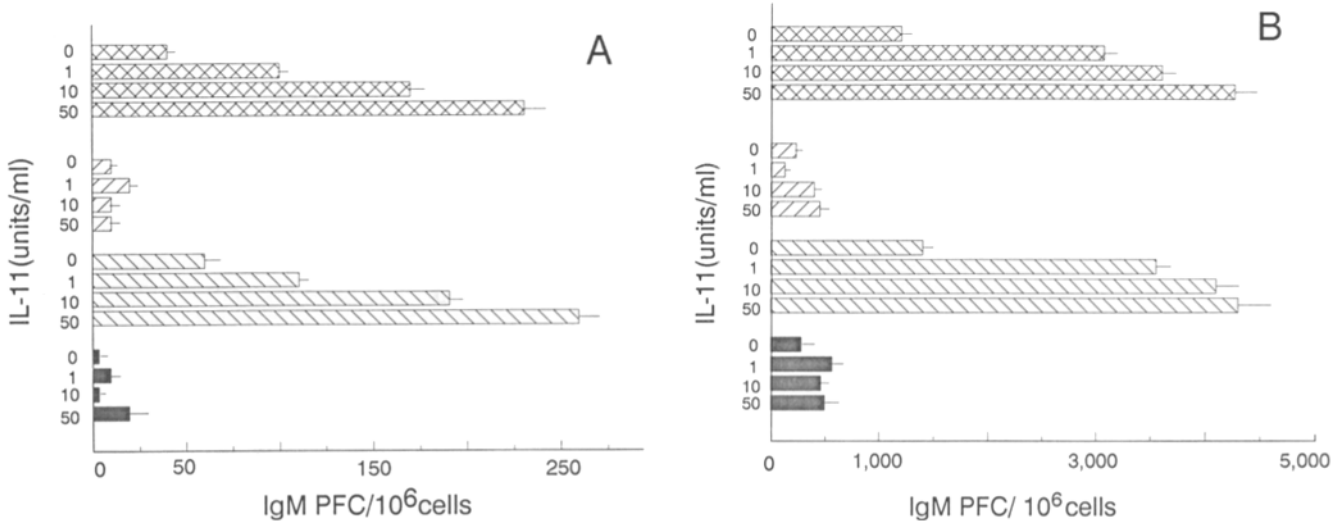

Figure 4. Effect of T cell depletion on rhuIL-11–induced augmentation of PFC response to SRBC in vitro. 2 × 10^6/well of spleen cells (▨) or spleen cells treated with anti-Thy-1.2 mAb (▨), anti-Lyt-2 mAb (▨) or anti-L3T4 mAb (■) plus complement, as described in Materials and Methods, were cultured with 10^6 SRBC for 5 d in primary response (*A*), or with 4 × 10^5 SRBC for 4 d in in vivo SRBC-primed spleen cell response (*B*). SRBC-specific direct PFC were determined and the mean (±SD) of duplicate cultures is shown. Two additional experiments showed similar pattern.

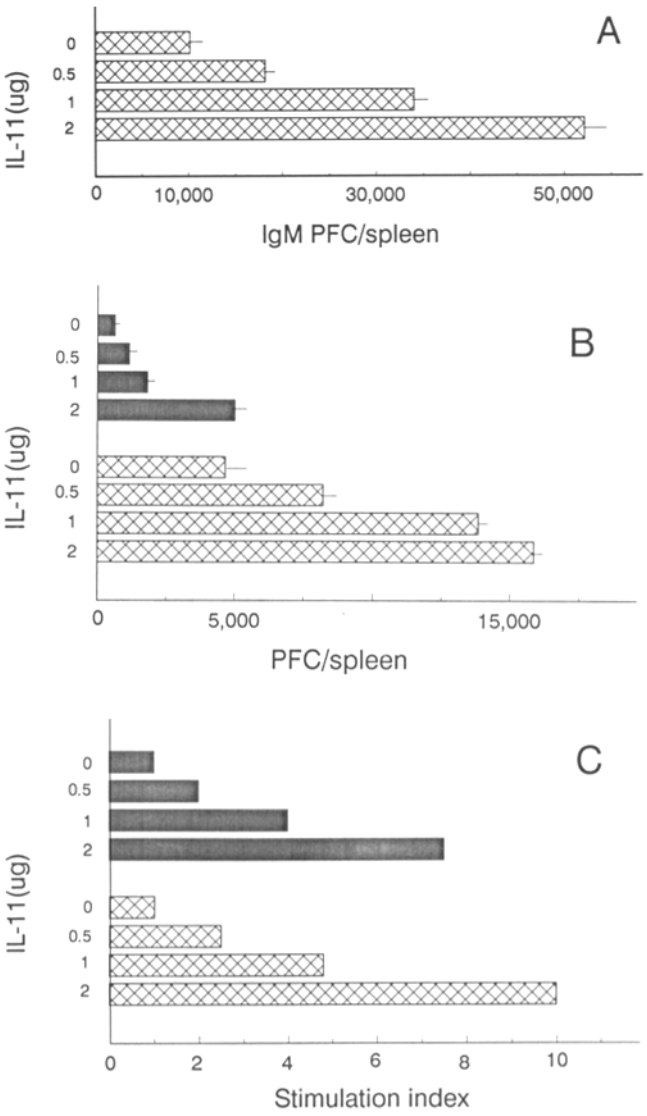

Figure 5. Effect of in vivo administration of rhuIL-11 on the primary and secondary responses. (*A*) Role of rhuIL-11 in primary response. C3H/HeJ mice were inoculated with 4 × 10^7 SRBC/mouse i.p. at day 0, and injected with indicated amounts of rhuIL-11 twice daily (once intraperitoneally, once subcutaneously) for 6 d. At day 7, mouse spleen cells were assayed for SRBC-specific direct PFC as described in Materials and Methods. The data are the arithmetic mean (±SD) of three individually assayed mice per group. Three additional experiments showed similar results. (*B*) Role of rhuIL-11 in secondary response. C3H/HeJ mice were injected with 4 × 10^7 SRBC/mouse i.p. 15 d after the first injection, mice were reinjected with 2 × 10^6 SRBC/mouse i.p. These mice were then injected with rhuIL-11 as indicated in *A*. At day 7, mouse spleen cells were assayed for SRBC-specific direct (■) and indirect (▨) PFC. The data are the arithmetic means (±SD) of three individually assayed mice per group. Two additional experiments showed similar results. (*C*) Role of rhuIL-11 in anti-SRBC antibody production in primary (■) and secondary (▨) response. Mice sera were obtained from the primary and secondary response to SRBC in vivo as indicated above. Serum anti-SRBC-specific antibody titers were then measured by hemagglutination test as described in Materials and Methods. The data expressed as stimulation index (defined as the anti-SRBC titer in treated mice/anti-SRBC titer in control mice) are the arithmetic means of three individually assayed mice per group. Two additional experiments showed similar results.

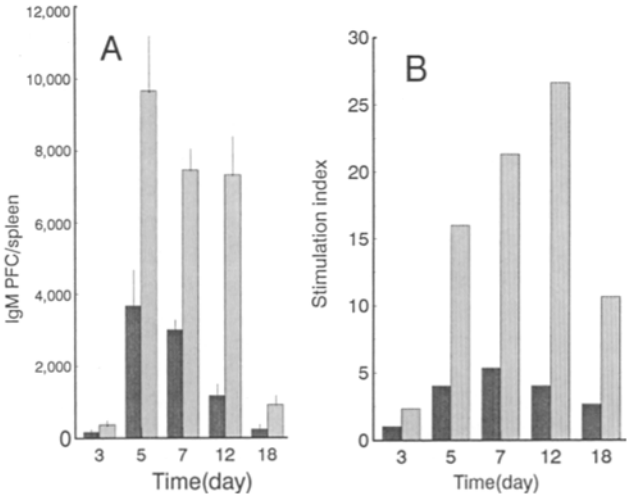

Figure 6. Effect of IL-11 administration on SRBC-specific antibody production in CYC-treated mice. 4 h after CYC treatment, C3H/HeJ mice were immunized with 4 × 10^7 SRBC i.p. On the next day, mice received IL-11 (▩) or control solution (■) injection for the duration of the experiment as described in Materials and Methods. (*A*) The number of spleen SRBC-specific direct PFC was determined and the results are the arithmetic means of three individually assayed mice per group for each time point. CYC-treated mice with IL-11 injection had significantly higher number of spleen SRBC-specific PFC when compared with CYC-treated mice without IL-11 treatment (day 3, $p < 0.02$; day 5, $p < 0.02$; day 7, $p < 0.01$; day 12, $p < 0.01$; day 18, $p < 0.02$). (*B*) Sera obtained from three mice per group were pooled, and anti-SRBC antibody titer was measured by hemagglutination test as described in Materials and Methods. The data are expressed as the stimulation index (defined as the anti-SRBC titer in control mice or in IL-11-treated mice/anti-SRBC titer in day-3 control mice).

on the enhancement of SRBC-specific PFC response by rhuIL-11.

Effect of Depletion of T Cells from Spleen Cells on rhuIL-11 Induced Enhancement of the PFC Response. To understand what cell populations in the spleen were required for the augmentation of the production of the SRBC-specific PFC by rhuIL-11, we tested the effect of T cell depletion on the IL-11–induced enhancement of SRBC-specific PFC. As shown in Fig. 4, depletions of Thy-1.2$^+$ or L3T4$^+$ cells dramatically decreased the rhuIL-11–induced enhancement of SRBC-specific PFC response using spleen cells primed with SRBC in vitro or in vivo. The depletion of Lyt-2$^+$ T cells did not affect the enhancement of PFC by rhuIL-11.

Effect of rhuIL-11 Administration on the Primary and Secondary Antibody Response in Normal Mice. Since rhuIL-11 enhances Ag-specific antibody responses in vitro, we next examined whether the administration of rhuIL-11 has similar effects in vivo. As shown in Fig. 5, *A* and *C*, administration of rhuIL-11 enhanced SRBC-specific PFC response and anti-SRBC antibody titer in a dose-dependent manner in the primary immune response. At the concentration of 4 μg/mouse/d, rhuIL-11 increased SRBC-specific PFC 5.2-fold and anti-SRBC antibody titer 7.5-fold, respectively. In the secondary immune response, mice were reinjected with 2 × 10^6 SRBC/mouse i.p. 15 d after the first injection of 4 × 10^7 SRBC/mouse.

RhuIL-11 injection and assay for PFC as well as serum antibody titer were determined as described in Materials and Methods. It was found that rhuIL-11 strongly enhanced the Ag-specific antibody response in a dose-dependent fashion (Fig. 5, *B* and *C*). At the concentration of 4 μg/mouse/d, rhuIL-11 increased SRBC-specific IgM PFC eight- and IgG PFC fourfold, and serum SRBC-specific antibody titer 10-fold, respectively. These results are comparable with the results obtained in the IL-6 system (6). However, the effects of IL-11 on Ag-specific antibody responses in the primary response in vivo (Fig. 5, *A* and *C*) may be different from those of IL-6 (6) and other cytokines. Both IL-1 (19) and IL-6 (6) have been suggested to act directly on B cells and T cells. TNF-α (20) may affect on T cells, and IL-3 (21) possibly acts on accessory cells. IL-11 may not directly affect B cells as evidenced by the in vitro studies. Whether IL-11 has direct effects on T cells or accessory cells remains to be clarified.

We also observed that administration of rhuIL-11 (4 μg/mouse/d for 6 d) did not significantly enhance the SRBC-specific PFC response or increase the total Ig content in mice that did not receive a secondary SRBC injection when compared with the control. Furthermore, SRBC-specific PFC in spleen and total Ig concentration in serum were not significantly enhanced by the administration of rhuIL-11 into normal mice without Ag stimulation. These results demonstrated that rhuIL-11 is not a polyclonal activator under our experimental conditions. However, we do not rule out the possibility that IL-11 may be a polyclonal activator at very high concentration since it has been reported that IL-6 is a very effective polyclonal activator in vivo when tested at high concentration (22, 23). These questions will be addressed by using the IL-11 transgenic mouse model.

Effect of rhuIL-11 Administration on Restoration of Impaired Antibody Responses in CYC-treated Mice. In addition to its ability to augment in vivo Ag-specific antibody response in normal mice, we also assessed the ability of rhuIL-11 to stimulate the regeneration of Ag-specific antibody-forming cells in C3H/HeJ mice with CYC-induced lymphopenia. As shown in Fig. 6, *A* and *B*, the spleen SRBC-specific PFC response and serum anti-SRBC antibody titer of the CYC-treated mice that received rhuIL-11 injection were significantly enhanced at all time points when compared with the control CYC-treated mice. For example, CYC-treated mice with IL-11 administration at day 12 had 4.5-fold higher spleen SRBC-specific PFC and sixfold higher serum anti-SRBC antibody titer than control mice without IL-11 injection. This result suggests that rhuIL-11 may play an important role in stimulating the recovery of impaired Ag-specific antibody response in CYC-treated mice and IL-11 may have important therapeutic potential specifically to stimulate the regeneration of decreased Ag-specific humoral immune responses induced by CYC treatment.

The major concern in these in vivo experiments is the possible role of contaminating endotoxin in the Ag-specific antibody responses. RhuIL-11 used in these studies contained <1.25 ng endotoxin/mg protein of rhuIL-11. This very low concentration of endotoxin did not affect the Ag-specific humoral immune responses in the endotoxin-resistant C3H/HeJ mice. Therefore, the possibility that endotoxin contaminant might account for the observed rhuIL-11 effect in increasing Ag-specific antibody responses in vivo can be reasonably excluded.

In conclusion, the results presented here demonstrated that rhuIL-11 is a novel cytokine involved in modulating Ag-specific antibody production in vivo and in vitro in normal mice. In addition, administration of IL-11 into mice after chemical-induced lymphopenia significantly stimulates the reconstitution of decreased Ag-specific antibody-forming cells in CYC-treated C3H/HeJ mice. Although the mechanism by which IL-11 exerts its action in vivo is not clear at the present time, these data suggest that rhuIL-11 may be potentially useful as an therapeutic agent in enhancing humoral immune response under certain clinical situations where accelerated lymphoid functions might be beneficial.

We thank Becki Robling for assistance in preparing the manuscript.

Yu-Chung Yang is a Scholar of the Leukemia Society of America.

Address correspondence to Yu-Chung Yang, Walther Oncology Center, Indiana University School of Medicine, 975 West Walnut Street, IB 540, Indianapolis, IN 46202.

Received for publication 21 August 1991 and in revised form 7 October 1991.

References

1. Howard, M., and W.E. Paul. 1983. Regulation of B cell growth and differentiation by soluble factors. *Annu. Rev. Immunol.* 1:307.
2. Kishimoto, T. 1985. Factors affecting B cell growth and differentiation. *Annu. Rev. Immunol.* 3:135.
3. Kishimoto, T., and T. Hirano. 1988. Molecular regulation of B lymphocyte response. *Annu. Rev. Immunol.* 6:485.
4. Teranishi, T., T. Hirano, B. Lin, and K. Onoue. 1984. Demonstration of the involvement of interleukin 2 in the differentiation of Staphylococcus aureus cowan I stimulated B cells. *J. Immunol.* 133:3062.
5. Splawski, J.B., L.M. McAnally, and P.E. Lipsky. 1990. IL-2 dependence of the promotion of human B cell differentiation by IL-6 (BSF-2). *J. Immunol.* 144:562.
6. Takatsuki, F., A. Okano, C. Suzuki, R. Chieda, Y. Takahara,

T. Hirano, T. Kishimoto, J. Hamuro, and Y. Akiyama. 1988. Human recombinant IL-6/B cell stimulating factor 2 augments murine antigen-specific antibody responses *in vitro* and *in vivo*. *J. Immunol.* 141:3072.

7. Finkelman, F.D., J. Holmes, I.M. Katona, J.F. Urban, Jr., M.P. Beckmann, L.S. Park, K.A. Schooley, R.L. Coffman, T.R. Mosmann, and W.E. Paul. 1990. Lymphokine control of *in vivo* immunoglobulin isotype selection. *Annu. Rev. Immunol.* 8:303.
8. Rasmussen, R. 1988. IL-5 is essential for antibody secretion by polyclonally and hapten specific activated B-cells. *J. Immunol.* 140:705.
9. Swain, S.L., R.W. Dutton, D. McKenzie, H. Helstrom, and M. English. 1988. Role of antigen in the B cell response: specific antigen and the lymphokine IL-5 synergize to drive B cell lymphoma proliferation and differentiation to Ig secretion. *J. Immunol.* 140:4224.
10. Van Snick, J. 1990. Interleukin-6: an overview. *Annu. Rev. Immunol.* 8:253.
11. Hilbert, D.M., M.P. Cancro, P.A. Scherle, R.P. Nordan, J. Van Snick, W. Gerhard, and S. Rudikoff. 1989. T cell derived IL-6 is differentially required for antigen-specific antibody secretion by primary and secondary B cells. *J. Immunol.* 143:4019.
12. Stevens, T.L., A. Bossie, V.W. Sanders, R. Fernandez-Botran, R.L. Coffman, T.R. Mosmann, and E.S. Vitetta. 1988. Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature (Lond.).* 334:255.
13. Go, N.F., B.E. Castle, R. Barret, R. Kastelein, W. Dang, T.R. Mosmann, K.W. Moore, and M. Howard. 1990. Interleukin-10, a novel B cell stimulatory factor: unresponsiveness of X chromosome-linked immunodeficiency B cells. *J. Exp. Med.* 172:1625.
14. Paul, S.R., F. Bennett, J.A. Calvetti, K. Kelleher, C.R. Wood, R.M. O'Hara, Jr., A.C. Leary, B. Sibley, S.C. Clark, D.A. Williams, and Y.-C. Yang. 1990. Molecular cloning of a cDNA encoding interleukin-11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proc. Natl. Acad. Sci. USA.* 87:7512.
15. Musashi, M., Y.-C. Yang, S.R. Paul, S.C. Clark, T. Sudo, and M. Ogawa. 1991. Direct and synergistic effects of interleukin-11 on murine hemopoiesis in culture. *Proc. Natl. Acad. Sci. USA.* 88:765.
16. Mishell, R.I., and R.W. Dutton. 1967. Immunization of dissociation spleen cell cultures from normal mice. *J. Exp. Med.* 126:423.
17. Dresser, D.W. 1986. Assays for immunoglobulin-secreting cells. *In* Handbook of Experimental Immunology. 4th ed. D.W. Weir, editor. Blackwell, Oxford. 64.1–64.26.
18. Luzuy, S., J. Merino, H. Engers, S. Izui, and P.H. Lambert. 1986. Autoimmunity after induction of neonatal tolerance to alloantigens: role of B cell chimerism and F1 donor B cell activation. *J. Immunol.* 136:4420.
19. Nencioni, L., L. Villa, A. Tagliabue, G. Antoni, R. Presentini, F. Perin, S. Silvestri, and D. Boraschi. 1987. *In vivo* immunostimulating activity of the 163-171 peptide of human IL-1β. *J. Immunol.* 139:800.
20. Ghiara, P., D. Boraschi, L. Nencioni, P. Ghezzi, and A. Tagliabue. 1987. Enhancement of *in vivo* immune response by tumor necrosis factor. *J. Immunol.* 139:3676.
21. Kimoto, M., V. Kindler, M. Higaki, C. Ody, S. Izui, and P. Vassalli. 1988. Recombinant murine IL-3 fails to stimulate T or B lymphopoiesis *in vivo*, but enhances immune responses to T cell–dependent antigens. *J. Immunol.* 140:1889.
22. Brandt, S.J., D.M. Bodine, C.E. Dunbar, and A.W. Nienhuis. 1990. Dysregulated interleukin-6 expression produces a syndrome resembling Castleman's disease in mice. *J. Clin. Invest.* 86:592.
23. Suematsu, S., T. Matsuda, K. Aozasa, S. Akira, N. Nakano, S. Ohno, J.-I. Miyazaki, K.-I. Yamamura, T. Hirano, and T. Kishimoto. 1989. IgG_1 plasmacytosis in interleukin-6 transgenic mice. *Proc. Natl. Acad. Sci. USA.* 86:7547.