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

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Summary

The availability of large quantities of highly purified recombinant interleukin 11 (rhIL-11) has allowed us to investigate the effects of rhIL-11 on sheep red blood cell (SRBC)-specific antibody responses in the murine system. The results showed that rhIL-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 rhIL-11 were abrogated completely by the addition of anti-rhIL-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 rhIL-11-stimulated augmentation of SRBC-specific antibody responses. The effects of rhIL-11 on the SRBC-specific antibody responses in vivo were also examined. RhIL-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, rhIL-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 (rhIL-11), like IL-6, can bring the pluripotent stem cells out of the G0 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 rhIL-11 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 rhIL-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.

Abbreviations used in this paper: Ag, antigen; CYC, cyclophosphamide; PFC, plaque-forming cells; rhIL-11, recombinant human interleukin 11.
Materials and Methods

Mice. C57BL/6 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.

RhIL-11. RhIL-11 produced in Escherichia coli and purified to homogeneity was obtained from Genetic Institute (Cambridge, MA). Purified rhIL-11 had a specific activity of 1.8 × 10^9 U/mg by T165 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-rhIL-11 antisera 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 × 10^6 to 10^9 SRBC in the presence or absence of various concentrations of rhIL-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 10% FCS, 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).

Effect of rhIL-11 on the Primary Immune Response to SRBC. We examined the effect of purified rhIL-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 rhIL-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, rhIL-11 induced IgM PFC response in a dose-dependent manner. The addition of as low as 1 U/ml rhIL-11 could increase IgM PFC 3.3-fold. At the concentration of 500 U/ml, rhIL-11 increased IgM PFC 6.7-fold. These results suggested that rhIL-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 rhIL-11 48 h after the initiation of spleen cell culture failed to increase Ag-specific PFC (data not shown), suggesting that rhIL-11, unlike IL-6 (6), has to be added at an early stage of cell culture in order to elevate Ag-specific PFC.

Results and Discussion

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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 × 10⁷ SRBC/mouse i.p. 2 d after immunization, spleen cells (2 × 10⁶/well in 0.5 ml medium) were restimulated with 4 × 10⁵ 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.

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.

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 × 10⁶/well in 0.5 ml medium) were cultured with 10⁶ 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 (fold in) 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.

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 × 10⁶/well of spleen cells (□□□□) or spleen cells treated with anti-Thy-1.2 mAb (■■■■), anti-Ly-2 mAb (□□□□) or anti-Ly-3 T4 mAb (□□□□) plus complement, as described in Materials and Methods, were cultured with 10⁶ SRBC for 5 d in primary response (A), or with 4 × 10⁵ 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.

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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 x 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).

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 re-injected with 2 x 10^6 SRBC/mouse i.p. 15 d after the first injection of 4 x 10^7 SRBC/mouse.
RhulL-11 injection and assay for PFC as well as serum antibody titer were determined as described in Materials and Methods. It was found that rhulL-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, rhulL-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 rhulL-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 rhulL-11 into normal mice without Ag stimulation. These results demonstrated that rhulL-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 rhulL-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 rhulL-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 rhulL-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 rhulL-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. RhulL-11 used in these studies contained <1.25 ng endotoxin/mg protein of rhulL-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 rhulL-11 effect in increasing Ag-specific antibody responses in vivo can be reasonably excluded.

In conclusion, the results presented here demonstrated that rhulL-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 rhulL-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.

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