

ADMINISTRATION OF RECOMBINANT INTERLEUKIN 2 IN
VIVO INDUCES A POLYCLONAL IgM RESPONSE

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IL-2 is a T cell-derived soluble factor with a broad spectrum of growth and differentiation-promoting activities (1). It provides a signal for cell proliferation by binding to high-affinity receptors on responsive cell populations. IL-2 receptors are expressed by activated T cells, and their functional importance has been shown in vivo (2). Administration of IL-2 in vivo specifically enhances allogeneic responses and allows induction of cytotoxic and helper T cells in nude mice and in cyclophosphamide-treated animals (3-5). The recent finding (6) of IL-2 receptors on B cells suggest that IL-2 might also exert a function as a B cell growth and differentiation factor. We wished to explore the functional role of IL-2 in the initiation and differentiation of a humoral immune response in vivo. IL-2 given in vivo could potentially affect humoral responsiveness by two different mechanisms: amplification of B cell responses by expansion of the helper cell pool, and/or by direct stimulation of B cells. We report here that administration of rIL-2 in vivo caused polyclonal IgM production. Treatment with rIL-2 clearly augmented the IgM production in primary as well as secondary immune responses, but it did not affect IgG secretion, nor did it induce a class switch from IgM to IgG in genetically low-responding mice.

Materials and Methods

Animals. BALB/c, A/J, A.BY mice and Swiss nude mice were bred in the animal facilities at Stanford University, or were purchased from The Jackson Laboratory, Bar Harbor, ME.

IL-2. rIL-2 was kindly supplied by the Cetus Corp., Emeryville, CA (lots LP222, LP236, and LP265B). Endotoxin concentration was <0.05 ng per 10^6 U IL-2, as measured in a Limulus assay. A buffer control from *E. coli* that did not contain the IL-2 insert but was equivalent in all other respects was used in the control mice.

Determination of Antibody Titers. Concentrations of antibodies in the sera were measured by solid-phase ELISA (7).

Estimation of Precursor Frequencies in Limiting-dilution Assays. Precursor frequencies of antigen-specific proliferative T cells were determined in a limiting-dilution system. Briefly, DBA/2 mice were immunized with 100 μ g soluble sperm whale myoglobin (SpWMB) at the base of the tail and injected with nine doses of 5,000 U rIL-2 i.p. for 3 d after immunization. Controls received excipient buffer instead of rIL-2. Regional draining lymph nodes were harvested on day 8 after immunization. Limited numbers of lymph

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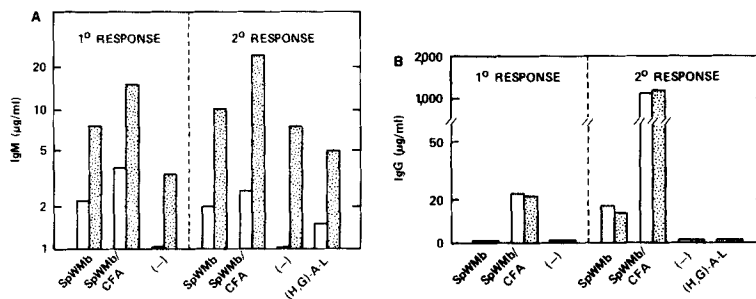


FIGURE 1. Effects of treatment with rIL-2 in vivo on humoral responses. BALB/c mice were either not immunized or immunized with 100 µg soluble SpWMB (Sigma Chemical Co., St. Louis, MO) or 100 µg SpWMB dissolved in CFA i.p. AB.Y mice received 100 µg (H,G)-A-L (a gift of Dr. H. O. M. McDevitt, Stanford University) in CFA. On day 14 mice were challenged with 50 µg aqueous antigen or 10 µg antigen in IFA. Mice were treated with either three daily doses of 5,000 U rIL-2 (shaded bars) or excipient control buffer (open bars), the three days after each immunization. SpWMB-specific IgM (A) and IgG concentrations (B) were measured on day 6 after the primary immunization, and on day 5 after the secondary immunization. Antibody concentrations to (H,G)-A-L were obtained on day 7 of the secondary response. Bars are mean antibody concentrations of 3–5 mice/group, SEM was <20%.

node cells (80,000–625 cells/well) were expanded in the presence of 10^6 syngeneic irradiated spleen cells and 10 µM SpWMB in flat-bottom, 96-well microtiter plates (Linbro, McLean, VA). 20 U/ml of rIL-2 were added at the beginning of the culture period. Microcultures were fed on day 7. On day 12, the cultures were washed three times and restimulated with fresh feeder cells and SpWMB in the absence of exogenous IL-2. Proliferation was assessed by [3 H]thymidine incorporation after 72 h. Individual cultures were scored positive when [3 H]thymidine incorporation exceeded background levels of control cultures by more than three standard deviations. Data were analyzed by Poisson analysis.

Results and Discussion

Treatment with rIL-2 In Vivo Induced Polyclonal IgM Production. To study the role of rIL-2 in a humoral immune response in vivo, mice were treated with rIL-2 for 3 d after immunization with a soluble antigen. Conventional immunization with soluble SpWMB induced the production of IgM antibodies in the primary response and a mixture of IgM and IgG antibodies in the secondary response (Fig. 1). When SpWMB was given in CFA, the animals produced IgM and IgG anti-SpWMB antibodies in the primary response and showed increased IgG concentrations after secondary antigen challenge (Fig. 1). Treatment with three daily doses of 5,000 U rIL-2 injected after immunization caused a 3–10-fold increase in the IgM titers to both antigens. Administration of rIL-2 did not influence IgG production in any of the experimental groups. Surprisingly, mice treated with rIL-2 alone without exogenous antigen also developed elevated titers of antibodies specific to SpWMB (Fig. 1a). Such antibodies induced by the rIL-2 injection were exclusively IgM. The second series of rIL-2 injections on days 15, 16, and 17 was again followed by the production of anti-SpWMB IgM antibodies. To exclude a possible nonspecific effect and to analyze the spectrum of IgM antibodies produced in rIL-2-treated mice, sera from rIL-2-treated mice were tested for their anti-KLH reactivity. All mice that received high doses of rIL-2 produced IgM (but not IgG) antibodies against KLH (Fig. 2). To analyze the specificity of the rIL-2-induced IgM antibodies, we absorbed pooled sera

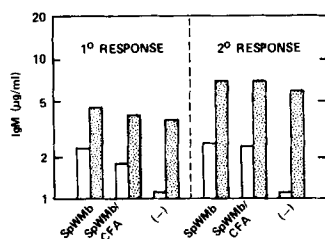


FIGURE 2. Humoral responses to unrelated antigen after treatment with rIL-2 or immunization with antigen. Anti KLH (Calbiochem-Behring, San Diego, CA) IgM antibodies were measured in BALB/c mice that were either not immunized, immunized with aqueous SpWMB, or with SpWMB in CFA. Mice were treated with control buffer (open bars) or with rIL-2 (shaded bars), and sera were collected, as described in Fig. 1. IgM antibodies against KLH were measured. Data represent the means of groups of five mice.

from five BALB/c mice on SpWMB-coupled plates. After repeated absorptions, the antibodies binding to SpWMB were removed without altering the titer of anti-KLH antibodies (data not shown). This result showed that antibodies with different specificities were present in sera of mice treated with rIL-2, and suggested the possibility that rIL-2 induced a polyclonal stimulation of IgM antibody production. Similar effects (i.e., three- to sixfold-increased antibody responses) were obtained when BALB/c mice were immunized with KLH and when A/J mice were given (H,G)-A-L followed by rIL-2 (data not presented).

Treatment with rIL-2 In Vivo Induced Polyclonal T Cell Activation. It is conceivable that rIL-2 primarily expanded the pool of antigen-specific T cells, and thus secondarily affected B cell function. To analyze the possibility of a direct effect of injected rIL-2 on T cells, a limiting-dilution system was established that allowed the estimation of precursor frequencies of SpWMB-specific proliferative T cells. In naive DBA/2 mice, 1 in 40,120 lymph node cells proliferated in response to SpWMB (Fig. 3). After a single immunization with SpWMB, the frequency of proliferative T cell precursors in regional draining lymph nodes increased ~3.5-fold to 1 in 11,625. Lymph nodes of rIL-2-treated animals contained as many SpWMB-specific proliferative T cells as did animals immunized with SpWMB (1 in 12,410). Injection of rIL-2 in vivo in addition to SpWMB, further increased SpWMB-specific proliferative T cells to a frequency of 1 in 4,530.

Immunization with Specific Antigen Induced a Polyclonal IgM Response. Activation of T lymphocytes by specific antigen is generally accompanied by the release of endogenous IL-2. To test the hypothesis that exogenous rIL-2 might mimic a physiological effect of locally released IL-2 we measured antibody titers against the unrelated antigen KLH in the sera of SpWMB immunized mice. Similar to results seen in rIL-2 treated mice, IgM antibodies specific for KLH were found after immunization with SpWMB (Fig. 2).

Potential Mechanisms of Action of rIL-2 In Vivo. In contrast to recently published data (8), we did not find an effect of rIL-2 on IgG antibody production, nor could we overcome Ir gene-controlled low responsiveness. Our observation that the injection of rIL-2 enhanced the production of antigen-specific antibodies might be explained by a direct effect of rIL-2 on the antigen-specific helper T cells. In our analysis of the T cell repertoire, similar frequencies of T cells responded to SpWMB in immunized and unimmunized rIL-2-treated mice.

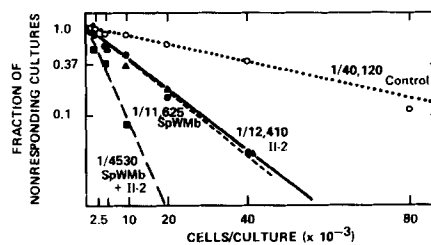


FIGURE 3. Precursor frequencies of antigen-specific proliferative T cells. BALB/c mice were immunized with 100 μ g aqueous SpWMB and were treated with either rIL-2 (■) or excipient control buffer (●) for 3 d after antigen injection. Control mice received rIL-2 (▲) or excipient control buffer (○) in the absence of any exogenous antigen. Draining lymph nodes were harvested on day 8 after immunization, and limiting-dilution analyses were performed as described in Materials and Methods.

However, the frequency of SpWMB-reactive T cells after administration of both antigen and IL-2 seemed additive (Fig. 3). Not only did we see this additive effect at the level of T cell precursor frequencies, but IgM antibodies were additionally enhanced in the presence of IL-2 and antigen compared with either antigen or IL-2 alone (Fig. 1). The implications of these findings are interesting but currently unexplained.

In studies reported in this paper, the effect of exogenous rIL-2 was restricted to an augmentation of IgM production suggesting that the adjuvant-like effect of rIL-2 is isotype selective. A random expansion of antigen-specific helper T cells might be expected to affect both IgM and IgG responses. However, as reported from our laboratory and elsewhere, helper T cells have two independent pathways of help (7, 9), and it is possible that rIL-2 activates only the IgM pathway. Alternatively, there might be a direct effect of high doses of rIL-2 *in vivo* on IgM-producing B cells. B cell responses are regulated by a number of antigen-nonspecific, genetically unrestricted factors derived from T cells (10). Recently (6), the expression of functional IL-2 receptors on activated B cells was shown using anti-Tac, an mAb against the IL-2 receptor molecule and by binding studies with purified radiolabelled IL-2. High doses of rIL-2 showed definite activity as a differentiation factor in IgM synthesis *in vitro* (11). Other studies (12) have suggested that IL-2 can function in B cell proliferation induction. The concentrations necessary for the B cell differentiative activity *in vitro* (100 U/ml) or for B cell proliferation were probably achieved transiently in our model. In our immunization protocol, the administration of exogenous rIL-2 might provide a proliferative signal and/or a terminal differentiation signal to selected B cells resulting in a high rate of IgM synthesis and secretion.

To test the hypothesis that rIL-2 may have a direct effect on B cells, we studied rIL-2 in three different T cell-depleted (deficient) animal models: the nude mouse, the thymectomized, irradiated, bone marrow-reconstituted mouse (ATxBm), and the T helper cell-depleted mouse (by repeated treatment *in vivo* with anti-L3T4 mAb). Swiss nude mice, which received a total dose of 45,000 U rIL-2 over 3 d produced equivalent amounts of IgM anti-SpWMB compared to Swiss *nu/+* controls (data not shown). The nude mouse may be not an optimal system to study T cell-independent effects. Residual T cell precursors might differentiate without thymic influence, especially in the presence of exogenous IL-2. Additional evidence that rIL-2 may directly stimulate B cells came from a

second model. Treatment of mice with 600 μg mAb against L3T4 completely inhibited their primary response to antigen (13), however, such L3T4-depleted mice produced normal levels of IgM in response to the administration of rIL-2. In contrast, ATxBm mice failed to produce antibody in response to rIL-2 administration (data not presented).

Though we cannot exclude the possibility that rIL-2 acts on T cells and secondarily induces polyclonal IgM secretion, the selective effect of rIL-2 on the secretion of IgM antibodies might reflect the direct activation of a subset of B cells. Ly-1 B cells have been described (14) as a functionally distinct B cell lineage. In autoimmune mouse strains, this B cell subpopulation contains the cells responsible for spontaneous IgM secretion, as well as those cells producing IgM autoantibodies. Characteristically, irradiated thymectomized mice are missing this B cell lineage when reconstituted with bone marrow cells (14). The inability of ATxBm mice to respond to the rIL-2 treatment support the possibility that Ly-1 B cells may play an important role in the observed phenomena. Additional support for this hypothesis comes from the demonstration that a subpopulation of the Ly-1 B cells contained in peritoneal exudate cells expressed a molecule identified by mAb against the IL-2 receptor (Weyand, C., P. Lolar, C. G. Fathman, and L. Herzenberg, unpublished observation).

IL-2 production and release into the local microenvironment seems to be a constant feature of every immune response. We do not know if such local IL-2 concentrations are comparable to the concentrations achieved by the administration of exogenous rIL-2 in this study. As the administration of rIL-2 alone triggered a humoral immune response against unrelated antigens, immunization with a specific antigen resulting in IL-2 production might be accompanied by a polyclonal IgM response. Indeed, we found elevated IgM titers against KLH in mice that had been immunized with SpWMB (Fig. 2).

The observed enhancement of the IgM response may be of therapeutic relevance. Recent observations in our laboratory suggests that pretreatment with rIL-2 can increase the survival of mice subsequently (1 wk later) challenged with an LD₅₀ dose of *E. coli*. (Weyand, C., J. Goronzy, P. O'Hanley, and C. G. Fathman, manuscript in preparation).

Summary

We studied the potential immunoenhancing effects of high doses of rIL-2 on murine T and B cell functions in vivo. Injection of rIL-2 caused a threefold or more increase in the frequencies of antigen-specific proliferative T cells, suggesting that rIL-2 initiated a polyclonal T cell response. In primary and secondary humoral immune responses, administration of rIL-2 in vivo selectively enhanced the production of IgM antibodies, whereas the IgG response was unaffected. Coadministration of rIL-2 with antigen failed to induce an isotype switch from IgM to IgG in genetically low-responding mice. Interestingly, in mice treated with rIL-2 alone (in the absence of exogenous antigen), polyclonal IgM production was induced. Polyclonal IgM production of lesser magnitude was found when mice were immunized with specific antigen in the absence of exogenous rIL-2, suggesting that local IL-2 concentrations in a primary immune response might be sufficient to elicit a polyclonal IgM response.

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