MONOClonAL ANTIBODY DIRECTED
AGAINST INTERLEUKIN 2

I. Inhibition of T Lymphocyte Mitogenesis and the In Vitro
Differentiation of Alloreactive Cytolytic T Cells

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Recent experimentation conducted in our laboratory described the generation of B
cell hybridomas whose antibody product neutralized interleukin 2 (IL-2) (formerly
called T cell growth factor) -dependent T cell replication (1). Three discrete experimental
arguments suggested that the inhibitory activity was associated with a
monoclonal IgG antibody directed against IL-2 determinants. First, passage and
subsequent acid elution of cloned hybrid cell culture supernate over a protein A-
Sepharose column yielded purified IgG fractions that inhibited mouse, rat, and
human IL-2 activity. Second, hybridoma-derived IgG in concert with lyopholized
Staphylococcus aureus (Igsorb) was capable of precipitating both "cold" and biosynthet-
ically radiolabeled IL-2 activity. Finally, Sepharose conjugated with purified anti-
IL-2 IgG provided an extremely active IL-2 absorption matrix (1).

Given the hypothesized role that IL-2 plays in mitogen-induced T cell proliferation
(2) and in the antigen-driven differentiation of cytolytic effector cells (3), we asked
whether monoclonal antibody directed against IL-2 would have a suppressive effect
on either (a) mitogen-stimulated T cell proliferation or (b) antigen-directed generation
of alloreactive cytolytic T cells (CTL). In this communication, we report that addition
of monoclonal anti-IL-2 IgG to cultures of concanavalin A (Con A)-stimulated or
alloantigen-activated murine splenocytes inhibited both mitogen-induced proliferation
and the in vitro generation of CTL. Furthermore, the capacity of anti-IL-2
antibody to inhibit T cell proliferation was enhanced by the presence of insolubilized
S. aureus. These results support the contention that IL-2 production is obligatory for
T cell mitogenesis and the in vitro generation of alloreactive CTL, and furthermore
suggest that antibodies to IL-2 may prove to be of significant value as immunosup-
pressive agents.

Materials and Methods

Production and Purification of Monoclonal Anti-IL-2 IgG. Spleen cells harvested from BALB/c
mice previously immunized with rat IL-2 were fused with the drug-marked myeloma SP-2.

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American Cancer Society.
Several of the resultant hypoxanthine-aminopterin-thymidine-resistant hybrid cell clones secreted a soluble product that significantly inhibited (>50%) IL-2-dependent T cell proliferation when tested in conventional T cell growth factor microassays (4). Of the potential anti-IL-2 secreting hybridomas, the clone designated 4E12B2H5 was selected for further characterization based on the capacity of this cell line's supernate to totally inhibit IL-2-dependent T cell line proliferation. In fact, when tested at a dilution of 1:20 in the presence of the co-precipitating matrix Igsorb (The Enzyme Center, Boston, Mass.), 4E12B2H5 culture supernate completely abrogated tritiated thymidine ([3H]TdR) incorporation of T cell lines as monitored after 24-h culture in the presence of 3 U/ml IL-2 (1).

Peritoneal ascites containing large concentrations of anti-IL-2 antibody were produced by intraperitoneal challenge of BALB/c female mice (6-8 wk of age, from the Central Animal Facility, Fred Hutchinson Cancer Research Center) with 2 x 10^6 4E12B2H5 hybridoma cells. Mice were challenged 1 wk after intraperitoneal injection with 0.5 ml of pristane (Baker Chemical Co., Seattle, Wash.). 10-14 d after the administration of 4E12B2H5 hybridoma cells, intraperitoneal ascites were harvested, clarified by centrifugation, and frozen (-20°C) until used for purification of anti-IL-2 IgG.

IgG purification was achieved by successive binding/elution from protein A-coupled Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.). 3-5 ml of hybridoma ascites was passed over a 10-ml protein A-Sepharose column equilibrated in 0.9% NaCl. Anti-IL-2 IgG was then removed from the column by elution with 200 mM glycine HCl buffer, pH 3. Purified antibody was then dialyzed overnight against 1,000 vol of 0.9% NaCl Hepes, pH 7.2, and frozen (-20°C) until use. The approximate antibody concentration was determined by absorption of ultraviolet light (280 nm) using a Beckman spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.), E_280 = 10. The capacity of the antibody to inhibit IL-2-dependent T cell line proliferation was assayed in 200 μl cultures containing 4 x 10^4 IL-2-dependent T cell line cells (CTLL-2) (5), 3 U/ml IL-2, and various concentrations of anti-IL-2 IgG. Replicate cultures (precipitating) also contained Igsorb at a final dilution of 1:200. Inhibition of proliferation was assessed by a 4-h pulse of [3H]TdR (20 Ci/mM, New England Nuclear, Boston, Mass.). [3H]TdR incorporation was assessed by liquid scintillation.

**Mitogenesis Assays.** The effect of anti-IL-2 on T cell mitogen-induced proliferation was tested in replicate 200 μl cultures (RPMI 1640, 2% fetal calf serum [FCS], 50 U/ml penicillin, 50 μg/ml streptomycin, and 300 μg/ml fresh L-glutamine) of C57BL/6 splenocytes (10^6 cells/ml) containing 2 μg/ml Con A and various concentrations of anti-IL-2 IgG in the presence and absence of Igsorb (final dilution of 1:400). As a further control, separate Con A stimulation cultures were initiated that contained an identical concentration of a monoclonal anti-gp70 antibody of the same IgG subclass. The latter was generously provided by Dr. R. C. Nowinski and Dr. Milton Tamm of the Tumor Virology Program of the Fred Hutchinson Cancer Research Center.

**Mixed Lymphocyte Culture (MLC) Generation of Alloreactive CTL.** The effect of anti-IL-2 on the generation of CTL was tested in 2 ml MLC (RPMI 1640, 2% FCS) containing 5 x 10^6 C57BL/6 responder spleen cells and 10^5 BALB/c irradiated (1,500 rad) splenic stimulator cells. Replicate MLC were conducted containing varying concentrations of either anti-IL-2 IgG or an irrelevant monoclonal IgG in the presence and/or absence of Igsorb (final dilution of 1:400). After 5 d of culture (37°C in a humidified atmosphere of 5% CO2 in air), viable effector cells were harvested and tested for their ability to lyse 51Cr-labeled allogeneic (H-2a) P815 mastocytoma target cells. Methods for performance of 4-h 51Cr release assays in 200 μl V-bottomed microwell plates have been described in detail elsewhere (6). Percent specific lysis was determined by using the following equation:

\[
\text{Percent Specific Lysis} = \frac{\text{cpm liberated in effector cell cultures} - \text{cpm liberated in background release culture}}{\text{cpm liberated in detergent lysis culture} - \text{cpm liberated in background release culture}} \times 100
\]

**Results**

**Anti-IL-2 Inhibition of T Cell Line Proliferation.** Fig. 1 reviews the ability of 4E12B2H5 hybridoma supernate, or purified IgG derived from ascites to inhibit the IL-2-driven...
Monoclonal Anti-IL-2 IgG Inhibition of T Cell Mitogenesis. Given (a) the capacity of monoclonal 4E12B2H5 antibody to inhibit IL-2-dependent T cell proliferation in both neutralizing and precipitating (in concert with IgSorb) cultures and (b) the hypothesis that IL-2 is the driving force behind normal mitogen-induced T cell proliferation (2, 7, 8), we were curious to determine what effect anti-IL-2 IgG would have on Con A-induced T cell mitogenesis. As shown in Table I, inclusion of anti-IL-2 IgG in cultures of Con A-stimulated normal murine spleen cells severely inhibited resultant T cell proliferation as assessed by $[^{3}H]$TdR incorporation during hours 72-76 in culture. Significant inhibition was observed with <1 μg/ml of 4E12B2H5 IgG. However, Con A stimulation conducted either in the absence of anti-IL-2 or in the presence of 20 μg/ml of anti-gp70 IgG resulted in splenocyte stimulation indices >25 times the background $[^{3}H]$TdR incorporation observed.

As was repeatedly seen in assays testing the effect of anti-IL-2 on T cell line proliferation, the ability of anti-IL-2 IgG to inhibit mitogenesis was dose-dependent and enhanced in IgSorb-containing cultures. The capacity of the IgSorb reagent to augment inhibition of mitogenesis parallels our previous observation using anti-IL-2 to inhibit the proliferation of an IL-2-dependent T cell line (1). Collectively considered, these data suggest that 4E12B2H5 hybridoma produces an anti-IL-2 antibody.
### Table I

**Effects of Anti-IL-2 on Con A-induced Spleen Cell Mitogenesis**

| Responder Cells | Con A | Antibody | IgG (1:400) | 
|-----------------|-------|----------|-------------| 
|                 |       |          | [H]Tdr incorporation cpm | 
|                  | 2 µg/ml |          |             | 
| C57BL/6         |       |          |             | 
| C57BL/6         |       |          |             | 
| C57BL/6         | +     | Anti-IL-2 (5 µg/ml) | 6,142 | 
| C57BL/6         | +     | Anti-IL-2 (2 µg/ml) | 5,985 | 
| C57BL/6         | +     | Anti-IL-2 (1 µg/ml) | 16,199 | 
| C57BL/6         | +     | Anti-gp70 (20 µg/ml) | 78,165 | 
| C57BL/6         |       |          |             | 
| C57BL/6         | +     | Anti-IL-2 (5 µg/ml) | 2,965 | 
| C57BL/6         | +     | Anti-IL-2 (2 µg/ml) | 1,938 | 
| C57BL/6         | +     | Anti-IL-2 (1 µg/ml) | 4,937 | 
| C57BL/6         | +     | Anti-gp70 (20 µg/ml) | 83,795 | 

* Values given are the mean of four replicate cultures. Standard deviation values were consistently < 10%.

with reactivity directed against determinants distal to the biologically active site of the lymphokine.

**Effect of Anti-IL-2 on the MLC Generation of Alloreactive CTL.** Based on the ability of anti-IL-2 IgG to inhibit T cell mitogenesis, we asked whether similar addition of 4E12B2H5 hybridoma antibody to MLC would result in decreased generation of cytolytic effector cell reactivity. As detailed in Fig. 2, addition of anti-IL-2 (at concentrations as low as 1 µg/ml) at the initiation of alloantigen-stimulated C57BL/6 spleen cell cultures markedly inhibited resultant effector cell reactivity as monitored 5 d later in 4-hour ⁵¹Cr release assays. As was observed in mitogenesis experimentation, the ability of anti-IL-2 to inhibit MLC-driven CTL differentiation was dose-dependent and was more effective in cultures conducted in the presence of a co-precipitating matrix (Fig. 2; Table II).

The data displayed in Table II also detail the deleterious effect that addition of 4E12B2H5 IgG had on the recovery of viable effector cells from MLC. Therefore, not only did anti-IL-2 serve to depress the lytic activity of CTL generated in MLC, but also significantly curtailed proliferation and recovery of responsive T cells. In contrast to the results obtained when cultures were supplemented with anti-IL-2 IgG, the addition of an irrelevant anti-gp70 immunoglobulin had no effect on the lytic reactivity of alloreactive CTL generated (even when tested at antibody concentrations as high as 40 µg/ml) or on viable cell recovery (Table II).

**Discussion**

The results presented in this communication have detailed the capacity of a monoclonal IgG antibody directed against a determinant present on IL-2 to severely inhibit several proliferation-dependent in vitro T cell immune responses. In addition to curtailing II-2-dependent T cell line proliferation, the addition of the 4E12B2H5 IgG reagent to spleen cell cultures markedly inhibited both Con A-induced T cell mitogenesis and alloantigen-induced generation of CTL. The ability of anti-IL-2 monoclonal antibody to inhibit these responses provides strong serological evidence that the production and use of IL-2 is essential for mitogen-induced T cell proliferation.
Viable effector cells harvested from 5-day MLC (C57BL/6 spleen cells stimulated in vitro with x-irradiated BALB/c splenocytes) were tested for lytic reactivity against 51Cr-labeled P815 (H-2d) tumor target cells. Responses detailed are those of effector cells harvested from control MLC, (O), MLC containing either 1 (△) or 5 (□) μg/ml of protein-A-Sepharose-purified anti-IL-2 IgG.

**Table II**

| X-irradiated effector cells | Anti IL-2 present | Percent yield | Percent specific lysis (P815) |
|-----------------------------|-------------------|---------------|-------------------------------|
|                             |                   |               | 50:1                          |
|                             |                   |               | 25:1                          |
|                             |                   |               | 10:1                          |
| C57BL/6 × BALB/c            | None              | 38            | 58                            |
| C57BL/6 × BALB/c            | 10 μg/ml          | 4             | 0                             |
| C57BL/6 × BALB/c            | 5 μg/ml           | 11            | 0                             |
| C57BL/6 × BALB/c            | 2 μg/ml           | 13            | 25                            |
| C57BL/6 × BALB/c            | 1 μg/ml           | 29            | 33                            |
| C57BL/6 × BALB/c            | 0.5 μg/ml         | 33            | 38                            |
| C57BL/6 × BALB/c            | Anti-gp70 present|               |                               |
|                             | None              | 47            | 60                            |
|                             | 40 μg/ml          | 44            | 59                            |
|                             | 20 μg/ml          | 52            | 54                            |
|                             | 10 μg/ml          | 51            | 58                            |
|                             | 5 μg/ml           | 45            | 59                            |
|                             | 2 μg/ml           | 53            | 58                            |

5-d MLC; 50:1 responder:stimulator ratio.

and for the in vitro differentiation of alloreactive CTL. The dose-dependent ability of anti-IL-2 IgG to inhibit CTL generation when assessed both in terms of resultant MLC cell recovery and/or lytic activity provides suggestive evidence that IL-2 not only drives the replication of antigen-primed effector cells but influences CTL differentiation as well.

Taken together with previous studies, which showed that only ligand-activated T cells could either absorb IL-2 or proliferate in response to in vitro stimulation with purified IL-2 (2, 7), the ability of antibody against IL-2 to inhibit T cell mitogenesis and the in vitro generation of alloreactive CTL argues forcibly in favor of the pivotal role that IL-2 plays in controlling T cell proliferation. In fact, it seems clear that antigen-dependent IL-2 production and use is what fuels ligand-activated T cell proliferation responses rather than the lectin or antigen itself.

Based on the data presented above, it would appear that in addition to being a potentially useful reagent for IL-2 radioimmunoassays and affinity purification of IL-2, anti-IL-2 IgG may be of importance in dissecting the involvement of IL-2 in a number of disease states, most notably hyperimmune syndromes and T cell malign-
nancy. In this regard it is interesting to note that the coupling of fluorescein to anti-IL-2 monoclonal antibody has resulted in the generation of an interesting reagent, which, when tested in cytoplasmic fluorescence assays, specifically stains only IL-2 producer cells (S. Gillis and D. Stull, unpublished observations). Finally, as indicated by its capacity to dramatically dampen mitogen-induced T cell replication and alloantigen-triggered CTL responses, it is conceivable that antibody directed against determinants present on the IL-2 molecule may function as an immunosuppressive drug. Such a reagent may be of value if indeed antigen-induced IL-2 production is a feature in aggravating hyperimmune disorders.

Summary

Our recent studies have detailed the generation of B cell hybridomas whose IgG product significantly inhibits interleukin 2 (IL-2)-dependent T cell replication. Given the capacity of such hybridoma antibody to interfere with the activity of mouse, rat, and human IL-2, we asked whether anti-IL-2 IgG would mediate similar inhibitory effects on other in vitro immune responses. In this communication, we report that addition of purified anti-IL-2 monoclonal antibody to either mitogen- or alloantigen-stimulated spleen cells exerted markedly deleterious effects on both resultant T cell proliferation and the generation of cytolytic effector cells. These results provide serological evidence in support of the integral role that IL-2 plays in controlling antigen/mitogen-induced T cell proliferation and serves further to define the ability of monoclonal antibody against IL-2 to function as an immunosuppressive agent.

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