Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6846–6850, November 1984
Immunology

Regulation of T-cell function by antibodies: Enhancement of the response of human T-cell clones to hepatitis B surface antigen by antigen-specific monoclonal antibodies

(immune regulation/antigen presenting cells/Fc receptors)

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Communicated by Herman N. Eisen, July 23, 1984

ABSTRACT **Eight mouse monoclonal antibodies specific for hepatitis B surface antigen (HBsAg) were examined for their effects on the antigen-induced proliferative response and lymphokine production of human HBsAg-specific T-cell clones *in vitro*. While all specifically enhanced the T-cell proliferative response, antibodies of the IgG class were generally more effective than those of the IgM class. Both the divalent $F(ab')_2$ and the monovalent Fab fragments of an IgG monoclonal antibody had no effects, indicating that the Fc portion of the antibody molecules was required. Since antigen-presenting cells bear surface receptors for the Fc of IgGs and fewer or none for that of IgMs, the above results also suggest that antibodies enhance the capture of antigens by antigen-presenting cells as a result of the binding of antigen–antibody complexes to the Fc receptors on these cells. In addition to potentiating the proliferation of the T-cell clones, antibodies also increased the antigen-induced production of interferon-γ by these cells. The present *in vitro* studies suggest that antibodies may regulate immune responses and do so by enhancing antigen presentation and thus augmenting antigen-induced activation and clonal expansion of T cells.**

The production of antibodies, generation of cytotoxic T lymphocytes, and development of other immune responses involve complicated interactions between different cell types and immune factors. T cells are known to play central roles shaping the outcome of most of these immune responses. Extensive studies of immunocytes in culture in recent years have shown that the activation and expansion of the antigen-specific regulatory T cells themselves are under numerous controls. The T cells are initially activated when they contact macrophages, reacting with antigens presented in association with the Ia antigens of macrophages (1–5). These T cells are further stimulated by interleukin 1 (IL-1) secreted by the activated macrophages (6–9) and their proliferation is sustained by one of their own secretory products, interleukin-2 (IL-2; see refs. 10 and 11). It is still unclear how many other factors are involved and how important they are in bringing about the effective functional regulatory T cells from their resting state.

We have used the *in vitro* response of human mononuclear cells to hepatitis B surface antigen (HBsAg) as a model system to analyze the numerous controls involved in the generation of antibodies and factors involved in the immune resistance to infectious diseases. We reported that the T lymphocytes present in the peripheral blood mononuclear cells (PBM) of hepatitis B vaccine recipients, but not of normal nonimmune individuals, proliferated *in vitro* when stimulated with purified HBsAg (12). Several T-cell clones reactive to HBsAg were isolated from the PBM of vaccine recipients by *in vitro* antigen stimulation in the presence of IL-2 (13). These cells proliferated specifically to HBsAg and produced increased amounts of interferon-γ (IFN-γ) and B-cell growth factor (36). Furthermore, at least two clones were found to be capable of promoting the *in vitro* production of antibodies to HBsAg (anti-HBs) by autologous B lymphocytes (13).

We have also studied the effects of antigen-specific antibodies, end products of the immune pathways, on the response of the regulatory T cells to the antigen. We observed earlier that purified anti-HBs from plasma of hepatitis B vaccine recipients specifically potentiated the antigen-induced proliferation of HBsAg-specific T-cell clones (14). We report here the effects of mouse monoclonal antibodies specific for HBsAg on the antigen-induced proliferation and IFN-γ production of human T-cell clones. We analyzed the effects of the different immunoglobulin classes and subclasses of the monoclonal antibodies and the requirement of the Fc portion of antibodies on the proliferative response of T cells to antigen. These studies may provide answers to the possible mechanism of the interesting antibody effect on T cells.

MATERIALS AND METHODS

Antigens. HBsAg-containing plasma was obtained from hepatitis B virus chronic carriers. Tissue culture supernatants containing recombinant DNA-derived HBsAg were kindly provided by Leslie B. Rall, Mary Anne Wormstead, and Pablo Valenzuela (Chiron, Emeryville, CA). The tissue culture-derived HBsAg (LSV-HBsAg) was synthesized by simian virus 40-transformed monkey kidney cells (Cos cells) expressing the LSV-HBsAg recombinant-DNA plasmid (15). HBsAg derived from human plasma and culture supernatant were purified by affinity chromatography using the mouse monoclonal IgM antibody H5D3 specific for HBsAg conjugated to agarose beads (Affi-Gel 10, Bio-Rad) as described (16). H5D3 was purified from ascitic fluid by chromatography on Sepharose 6B (Pharmacia). A different plasma-derived HBsAg preparation treated with pepsin and urea was furnished by William J. McAleer (Merck Sharp & Dohme). The pepsin/urea-treated HBsAg was purified from 22-nm HBsAg particles isolated from plasma pools by ammonium sulfate concentration and ultracentrifugation. The noninfectious 22-nm HBsAg particles were then treated with pepsin at 1 mg/liter (pH 2.1) at 37°C for 78 hr followed by 8 M urea for 4 hr to remove the associated human serum components (17). Protein content of all preparations was estimated by the Bio-Rad protein assay. As we previously reported (13), the HBsAg purified directly from plasma by affinity chromatog-

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Abbreviations: IL-2, interleukin 2; HBsAg, hepatitis B surface antigen; PBM, peripheral blood mononuclear cells; IFN-γ, interferon-γ; anti-HBs, anti-HBsAg antibodies.

raphy with H5D3 contained large amounts of human serum proteins tightly complexed to it. On the other hand, the pepsin/urea-treated HBsAg and LSV-HBsAg appeared to be free of serum proteins (<0.1 ng per 10 μg of HBsAg) as measured by sensitive radioimmunoassays.

Antibodies. Mouse monoclonal anti-HBs were prepared by somatic cell hybridization techniques as described (18). The mouse monoclonal antibody B2TT, specific for tetanus toxoid, was also used (19). Ascitic fluids containing 5–20 mg of specific antibodies per ml were used in most experiments. The amount of specific anti-HBs in each ascitic fluid was determined by a radioimmunoassay using HBsAg-coated polyvinyl 96-well plates, and ^{125}I-labeled goat anti-mouse IgG or goat anti-mouse IgM. The antigen binding activity in the ascitic fluids was compared with standard curves using purified monoclonal anti-HBs IgG (A5C3) or monoclonal anti-HBs IgM (H5D3). The monoclonal anti-HBs A5C3 (IgG2a) was purified from ascitic fluid by affinity chromatography on protein A Sepharose (Sigma). F(ab')$_2$ and Fab fragments of A5C3 were prepared by digestion with pepsin and papain, respectively, as described (20, 21). The F(ab')$_2$ and Fab fragments of A5C3 were further purified by removing the undigested IgG molecules and Fc fragments with protein A Sepharose. High pressure liquid chromatography analysis of the whole A5C3 antibody molecules and fragments revealed one single peak of the appropriate molecular weight for each preparation.

HBsAg-Specific T-Cell Lines and Clones. The detailed procedures for the isolation of HBsAg-reactive T-cell lines (HBL) and clones (HBC) have been published elsewhere (13). Briefly, 5 × 10^5 PBM per ml from hepatitis B vaccine (Hepatavax B, Merck Institute of Therapeutical Research, West Point, PA) recipients were stimulated in culture with plasma-derived HBsAg (20 μg/ml) for 6 days. Live cells were then resuspended at the original cell concentration in the presence of 10% T-cell growth supplement (TCGS, Meloy Laboratories, Springfield, VA) as a source of IL-2. After 6 additional days of incubation, the cells were resuspended at 1 × 10^5 per ml and restimulated with HBsAg at 20 μg/ml in the presence of 5 × 10^5 cells per ml of autologous-irradiated (2000 Rad) PBM and 5% TCGS. Clones were obtained by plating the cells 1 week later at limiting dilutions (1 cell per well) in 96-well flat-bottom plates (Costar, Cambridge, MA) in the presence of plasma-derived HBsAg (20 μg/ml)/20% fetal calf serum/autologous irradiated PBM (5 × 10^5 cells per ml)/10% TCGS. Tissue culture medium routinely consisted of RPMI 1640 (GIBCO) supplemented with 10% fetal calf serum (HyClone, Logan, UT)/2 mM L-glutamine (Sigma)/25 mM Hepes (Sigma)/gentamycin (50 μg/ml) (HEM Research, Rockville, MD)/50 μM 2-mercaptoethanol (Sigma). All tissue culture incubations were done at 37°C in 5% CO_2/95% humid air.

Cell Proliferation Assays. HBsAg-specific T cells (2 × 10^4) were mixed with autologous-irradiated PBM (1 × 10^5) in the presence of different concentrations of HBsAg and antibodies in a final vol of 200 μl of tissue culture medium for each well of 96-well flat-bottom well trays. The proliferation assays were incubated for 3 days and each culture well was pulsed for the last 15–20 hr with 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) (New England Nuclear). The cultures were harvested onto glass fiber filters, washed using a Skatron cell harvester (Sterling, VA), and the amount of radiolabel incorporated into DNA was determined by liquid scintillation spectroscopy. Results of cell proliferation were expressed as the mean cpm of [^{3}H]thymidine incorporated by duplicate cultures. The standard errors of the mean were always <10% of the value of the mean.

Production and Determination of IFN-γ. HBsAg-reactive T cells were incubated at 1 × 10^5 cells per ml with autologous-irradiated PBM at 5 × 10^5/ml in the presence and absence of anti-HBs and HBsAg for 48 hr in 24-well flat-bottom well trays (Costar). Supernatants were removed, filtered through a 0.2-μm pore membrane, and assayed for IFN-γ using an immunoradiometric assay developed in our laboratory (22). Quantitation of IFN-γ was done using a standard curve with DEAE-purified IFN-γ derived from mitogen-activated human PBM. The sensitivity of this assay was ≈0.1 NIH unit of IFN-γ per ml.

RESULTS

Eight mouse monoclonal antibodies specific for HBsAg were examined for their effects on the antigen-induced proliferation and immune factor production of three human HBsAg-specific T-cell clones. Two of these clones, HBC-6 and HBC-13, were shown to promote the production of anti-HBs by autologous B cells. The other two clones, HBC-1 and HBC-4, were also OKT4$^+$ OKT8$^-$, but their functions are not yet known (13). Fig. 1 shows the results obtained with a constant concentration (1 μg/ml) of these monoclonal anti-HBs on the proliferative response of a helper T-cell clone to different antigen concentrations. All of the monoclonal anti-HBs of IgG class (three IgG1 and two IgG2a) significantly increased the proliferation of HBC-13 to HBsAg. However, the effect of monoclonal anti-HBs of IgM class was far less dramatic than that of the antibodies of IgG class. Of the three monoclonal IgM anti-HBs tested, two increased the HBsAg-induced proliferation to a small extent at high antigen concentrations. A monoclonal anti-tetanus toxoid IgG did not affect the HBsAg-induced proliferation of the T-cell clone.

In a separate experiment using HBC-6 another helper T-cell clone, six of the monoclonal anti-HBs were studied at different concentrations for their effect on the antigen-in-

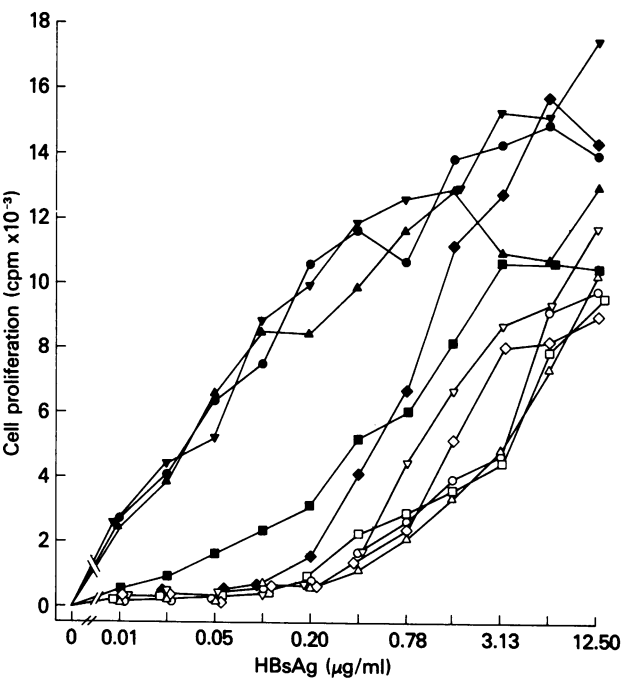

FIG. 1. Effects of monoclonal antibodies in the HBsAg-induced proliferation of T-cell clone HBC-13. Cell proliferation was determined with different concentrations of HBsAg and a constant concentration of each antibody (1 μg/ml). The HBsAg used in this experiment was derived from plasmas of chronic HBV carrier and contained high amounts of serum proteins. The monoclonal anti-HBs used were as follows: ◆, H4G7 (IgG1); ■, H2C4 (IgG1); ▲, A2C6 (IgG1); ●, A5C3 (IgG2a); ▼, A5C11 (IgG2a); ◇, H2F11 (IgM); □, H5D3 (IgM); ▽, H2F8 (IgM). The monoclonal anti-tetanus toxoid antibody B2TT (IgG2a) was included as a negative control (○) and proliferation in the absence of antibody was also determined (△).

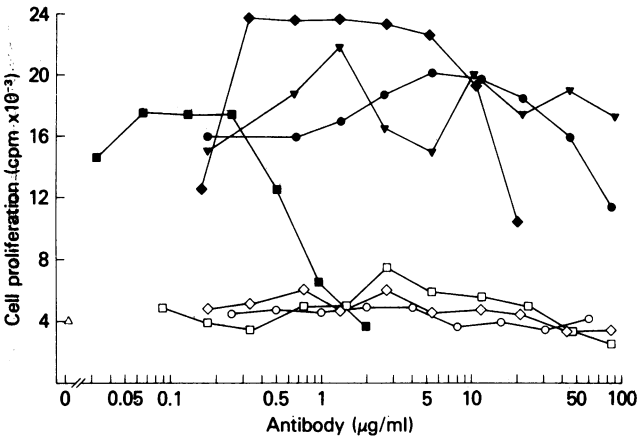

FIG. 2. Effects of different concentrations of monoclonal antibodies in the HBsAg-mediated proliferation of T-cell clone HBC-6. Antigen-induced proliferation was measured with different concentrations of antibodies and a constant concentration of HBsAg (1 μg/ml) in one single experiment. The monoclonal anti-HBs used were as follows: ◆, H4G7 (IgG1); ■, H2C4 (IgG1); ●, A5C3 (IgG2a); ▼, A5C11 (IgG2a); □, H5D3 (IgM); ◇, H2F11 (IgM). The monoclonal anti-tetanus toxoid antibody B2TT (IgG2a) was included as a negative control (○) and proliferation in the absence of antibody was also determined (△).

duced proliferative response at a constant concentration of HBsAg (1 μg/ml). The titration curves presented in Fig. 2 show that the four monoclonal IgG anti-HBs (two IgG1 and two IgG2a) tested were effective in enhancing the proliferation of the T cells to HBsAg over a wide range of antibody concentrations. On the other hand, in this experiment, no significant enhancement of the HBsAg-induced proliferation was observed at any concentration of the monoclonal anti-HBs of IgM class tested. As with HBC-13, the anti-tetanus toxoid IgG mouse monoclonal antibody had no effect on the antigen-induced proliferation of this T-cell clone.

The potentiating effect of one of the (IgG1) monoclonal anti-HBs on the antigen-induced proliferation of cloned T cells was also examined using two purer preparations of

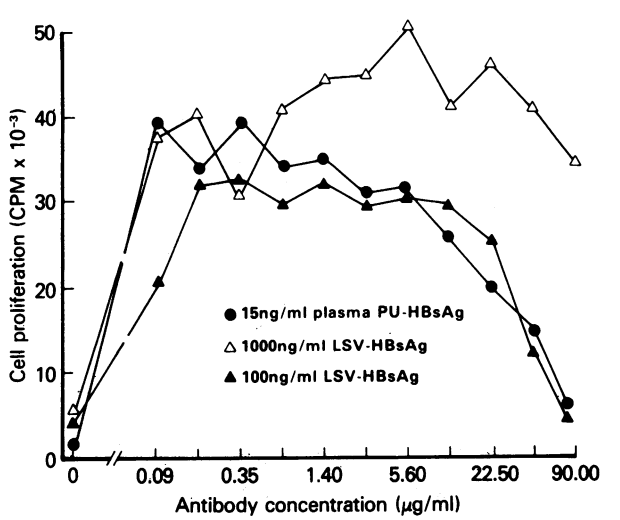

FIG. 3. Comparison of different HBsAg preparations on the potentiating effect of a monoclonal anti-HBs of the antigen-induced proliferation of a T-cell clone. Different concentrations of the mouse monoclonal anti-HBs A2C6 (IgG1) were tested for their capacity to enhance the proliferation of clone HBC-6 to a constant concentration of two different HBsAg preparations. PU-HBsAg, pepsin/urea-treated HBsAg from plasma; LSV-HBsAg, obtained from tissue culture supernatant of a recombinant DNA-derived cell line. Both antigens were free of human serum proteins.

Table 1. The effects of a mouse anti-HBsAg monoclonal antibody on the HBsAg-induced production of IFN-γ by a T-cell line and three T-cell clones

	IFN-γ produced, units/ml*					
	PU-HBsAg†		LSV-HBsAg‡		Medium	
T cell	−Ab	+Ab§	−Ab	+Ab	−Ab	+Ab
HBC-1	2.5	9.17	0.66	9.79	0.23	0.30
HBC-4	14.83	55.59	2.38	39.35	0.24	0.58
HBC-6	2.99	13.13	0.20	9.00	0.09	0.16
HBL¶	18.87	69.00	40.50	113.74	5.59	13.56

*NIH units/ml measured as described in *Materials and Methods*.
†Pepsin/urea-treated HBsAg (1 μg/ml) from human plasma.
‡HBsAg produced by recombinant DNA technology (1 μg/ml).
§Monoclonal IgG1 anti-HBs A2C6 (1.8 μg/ml).
¶HBL is the HBsAg-reactive T-cell line from which the clones were derived.

HBsAg, which do not contain human serum proteins. One preparation (pepsin/urea-treated HBsAg) was also derived from plasma of chronic carriers but was treated with pepsin and urea to remove the complexed human serum proteins and it is very similar to the Hepatavax-B (Merck Sharp & Dohme) vaccine (17). The other HBsAg preparation (LSV-HBsAg) was purified from the culture supernatant of a genetically engineered cell line that harbored and expressed DNA encoding HBsAg. The results presented in Fig. 3 show that the monoclonal IgG anti-HBs was also effective in enhancing the proliferation of HBC-6 cells to both of these HBsAg preparations.

The effects of anti-HBs on the antigen-induced production of IFN-γ, an important immunoregulatory and effector lymphokine, by HBsAg-specific T cells were also examined. The data presented in Table 1 indicate that, in all cases, significantly more IFN-γ was secreted by the three HBsAg-specific T-cell clones and by one HBsAg-specific T-cell line when both an IgG anti-HBs and HBsAg were present in the cultures than when antigen alone was used. In the absence of antigen, the anti-HBs slightly increased the production of IFN-γ.

The role of the Fc portion of the antibody molecule in the potentiation of the response of T cells to HBsAg was addressed. Fig. 4 shows that only intact anti-HBs IgG molecules were capable of enhancing the HBsAg-induced proliferation of HBC-6 cells. Both the $F(ab')_2$ and Fab fragments

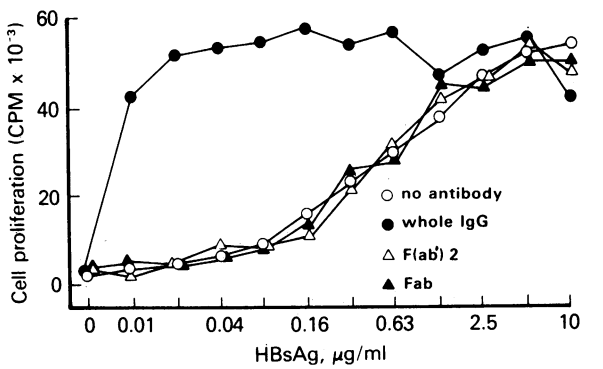

FIG. 4. Requirement for the presence of the Fc portion of the antibody molecule for the enhancement of HBsAg-mediated proliferation of an antigen-specific T-cell clone. The reactivity of clone HBC-6 to different concentrations of plasma-derived HBsAg was measured in the presence of constant amounts (1 μg/ml) of the purified monoclonal anti-HBs A5C3: whole IgG molecules (●), $F(ab')_2$ fragments (△), or Fab fragments (▲). Proliferation was also determined in the absence of antibodies (○). The HBsAg preparation used in this experiment was the same as that used in Figs. 1 and 2.

had no effect at all in the proliferative response of HBC-6 cells to all concentrations of HBsAg.

DISCUSSION

The experiments reported in this paper provide additional results demonstrating that antibodies serve not only effector functions in binding antigens but also regulatory functions on the expansion of antigen activated T-cell clones. From our earlier observation that human polyclonal anti-HBs were capable of increasing the antigen-induced proliferation of HBsAg-reactive human T cells (14), we suggested a few mechanisms to account for the effect of antibodies in potentiating the activity of antigen. Antibodies can polymerize antigens and increase their valence, which may enhance the binding affinity of surface receptors to the antigen (23). The aggregated antigen–antibody complex may also be more easily captured by phagocytic antigen-presenting cells (24). Furthermore, ingestion of immune complexes by the antigen-presenting cells may be facilitated by their binding to Fc and complement receptors on the surface of those cells (24–26). Some early studies by Cohen *et al.* (27, 28) showed that sera from immune animals enhanced the proliferation of guinea pig peritoneal exudate lymphocytes to antigen *in vitro*.

The present studies were designed to explore the mechanisms of this effect of antibodies on T-cell activity. The results indicate that all of the monoclonal anti-HBs of IgG class studied increased the antigen-induced proliferation of HBsAg-specific T-cell clones (Figs. 1 and 2). No significant difference was observed between the anti-HBs of IgG1 and IgG2a. This antibody effect also occurred with purer HBsAg preparations that did not contain serum proteins (Fig. 3). The IgM monoclonal anti-HBs were in general not as effective in enhancing the proliferative responses of the T cells to HBsAg (Figs. 1 and 2). Finally, our results also showed clearly that the Fc portion of the IgG anti-HBs was absolutely required for the antibodies to potentiate the proliferation of the cloned T cells to HBsAg (Fig. 4).

Since IgM antibodies should aggregate or polymerize antigens as well as or even better than IgG antibodies, and since the divalent $F(ab')_2$ fragments of IgG should still aggregate antigens like the intact IgG molecules, the above results suggest that aggregation of antigens *per se* was not the major factor accounting for the ability of the antibody to enhance the antigen-induced T-cell proliferation. The results do suggest that the Fc receptors on antigen-presenting cells, such as monocytes, were involved. The antibody–antigen complexes bind to Fc receptors on the antigen-presenting cells, and the $F(ab')_2$ fragments lose the ability to do so because the Fc portion has been cleaved. If this suggestion is correct, the Fc receptors for IgGs must be more abundant or more effective than those for IgMs or, alternatively, the antigen-presenting cells with IgG Fc receptors should be more numerous than those with IgM Fc receptors. In fact, the presence of Fc receptors for IgG1 and IgG2 on some antigen-presenting cells has been well established, but that for IgM still remains controversial (24–26, 29). The potentiating effect of antibodies on antigen-induced proliferation is not only restricted to HBsAg, and it probably occurs generally with a variety of antigens. We recently observed that both human polyclonal and mouse monoclonal anti-tetanus toxoid antibodies were effective in enhancing the antigen-induced proliferative response of human T cells specific for tetanus toxoid (unpublished results).

Although antigen-induced proliferation appears to be an adequate way of measuring T-cell activation, it does not measure T-cell effector or regulatory functions. The finding that monoclonal IgG anti-HBs also increased significantly the amounts of IFN-γ secreted by three HBsAg-reactive T-cell clones and one line upon antigenic stimulation (Table 1) indicates that not only proliferation but also effector function of T cells can be modulated by these antibodies.

The present observations suggest that antibody is an important element involved in the regulation of immune responses. It does so by potentiating the antigen-induced expansion of T-cell clones and the generation of effector functions. Our results from the *in vitro* studies may provide an explanation for the effects of antibodies in the immune response in whole animals. Numerous reports have appeared in the past two decades describing the effects of immune complexes and passively administered antibodies in potentiating or inhibiting *in vivo*-generated immune responses to some antigens (30–32). For example, Terres and co-workers (33, 34) observed that the injection of tetanus toxoid together with anti-tetanus toxoid antibodies in mice elicits a better immune response than the injection of antigen alone. Although the cellular or molecular targets of these immunoregulatory antibodies were not clearly determined, it was suggested 20 yr ago by Eisen and Karush (35) that bivalent antibodies determine immune responses or tolerant states *in vivo* through regulating the uptake of antigen by immunologically competent cells. It is conceivable that the synergistic effects of antibody on antigen-induced T-cell expansion observed *in vitro* could also occur *in vivo*. The enhancement or inhibition of an immune response *in vivo* could be the result of preferential expansion of helper, inducer, or suppressor T cells.

We thank Richard W. Miller and Gwen A. Melincoff for their excellent technical assistance and Sandra A. O'Haire for typing the manuscript.

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