IN VIVO EFFECTS OF ANTIBODIES TO IMMUNE RESPONSE GENE PRODUCTS

I. Haplotype-specific Suppression of Humoral Immune Responses with a Monoclonal Anti-I-A*

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The *I* region of the murine major histocompatibility complex (H-2) controls a number of critical immunologic functions, including genetic control of immune responses (*Ir* genes)[1] (1) and immune suppression (2), cooperation between macrophages and T cells (3) and between T and B cells (4), production of soluble helper and suppressor factors (5, 6), and synthesis of cell surface glycoproteins, known as Ia antigens, on B cells and subsets of T cells and macrophages (7). Ia antigens appear to be the molecules determining genetic control of immune responsiveness, at least in part by determining the way in which macrophages "present" antigen to T cells (3). *Ir* genes were initially defined in experiments using the branched, multichain synthetic polypeptide antigens (T,G)-A--L [(tyrosine, glutamic acid)-alanine--lysine] and (H,G)-A--L [(histidine, glutamic acid)-alanine--lysine] (8). Mice of the H-2^k haplotype can respond to (H,G)-A--L but cannot respond to (T,G)-A--L, whereas mice of the H-2^b haplotype can respond to (T,G)-A--L but not to (H,G)-A--L (8). The *Ir* genes controlling immune responsiveness to these antigens map to the *I-A* subregion (9, 10).

Anti-Ia antisera and monoclonal antibodies specific for *I*-region products have been useful in defining the roles of *I*-region gene products in in vitro immune response assays, including antigen presentation to T cells and primary and secondary antibody responses (10–14). Several reports indicate that these antibodies can modify in vivo immune responses including tumor and graft rejection, T cell helper activity and granuloma formation (15–22). In this study, we examined the effect of administering an anti-I-A^k monoclonal to H-$2^{k/b}$ heterozygous F_1 mice. The data indicate that, in certain experimental conditions, an anti-I-A^k monoclonal antibody can markedly suppress antibody production to (H,G)-A--L while minimally affecting (T,G)-A--L responses.

These results suggest that anti-*I* region monoclonal antibodies might be useful in haplotype-specific suppression of humoral responses to antigens under *Ir* gene control. If this type of immunosuppression can be extended to disease models under *Ir* gene

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[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; *Ir*, immune response; NMS, normal mouse serum; PBS, phosphate-buffered saline.

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control, a similar approach using haplotype-specific human monoclonal antibodies might be applicable to human autoimmune diseases in which susceptibility is linked to HLA-D or DR genotype. This type of immunosuppression offers the great practical advantage that the immune response to antigens not under the control of the *I* region product recognized by the monoclonal antibody would be relatively unaffected.

Materials and Methods

Mice. (C3H/DiSn × CWB/13Hz)F_1 mice were bred in our mouse colony at Stanford University. Mice of both sexes between the ages of 2–10 mo were used. In individual experiments, both control and experimental groups were matched for age and sex.

Antigen, Immunization Procedures, and Monoclonal Antibody. (T,G)-A--L 52 and (H,G)-A--L 905 were a gift from Dr. Michael Sela, Department of Clinical Immunology, The Weizmann Institute of Science, Rehovot, Israel. Preparation of these antigens has been described elsewhere (23). For experiments using adjuvant, mice were immunized in the hind footpads with 10 μg antigen emulsified in complete Freund's adjuvant (CFA) containing 2 mg *Mycobacterium tuberculosis* cells per ml (Difco Laboratories, Detroit, Mich.), followed 3 wk later with the same dose of antigen in phosphate-buffered saline (PBS) intraperitoneally. The mice were bled 8 d later. Mice receiving antigen in aqueous solution were immunized with 100 μg (T,G)-A--L or (H,G)-A--L in PBS i.p., followed 8 d later with the same antigen dosage injected i.p., and were bled 8 d after the secondary immunization, as described by Grumet (24).

The anti-I-A^k monoclonal hybridoma (H10-3.6) used in these experiments was provided by Dr. Patricia Jones, Dr. Vernon Oi, and Dr. Leonard Herzenberg. This antibody reacts with public specificity Ia.17, which is associated with H-$2^{k,r,s,f}$ (25). The hybridoma was maintained in ascites form, and the ascitic fluid had a titer $>10^4$ in a cell binding assay using H-2^k spleen cells as targets. Ascites was injected into the peritoneal cavity of H-$2^{k/b}F_1$ mice according to schedules described in Results.

Antibody Determinations. The humoral response of F_1 mice was determined by a solid-phase antigen binding assay similar to the allotyping assay as described by Tsu and Herzenberg (26). Briefly, polyvinyl chloride microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 30 μl of a solution of (T,G)-A--L or (H,G)-A--L (50 μg/ml in PBS) for 1 h. After removing unbound antigen and washing with PBS containing 5% newborn calf serum, 20 μl of a series of dilutions of mouse serum was added to each well and incubated for 1 h. Bound serum antibodies were identified with a ^{125}I-labeled rabbit anti-mouse immunoglobulin reagent. After 1 h, the plates were washed and the wells cut out and counted in a gamma counter. All dilutions were tested in duplicate, and the data are expressed as cpm bound by each serum dilution. Normal mouse serum (NMS) served as the background control. A 1:10 dilution of NMS bound between 150 and 300 cpm.

Results

As depicted in Figs. 1 and 2, (H,G)-A--L-immunized (C3H × CWB)F_1 mice treated in vivo with an anti-I-A^k monoclonal antibody had a significant reduction in their humoral responses to this antigen. (C3H × CWB)F_1 mice immunized with (T,G)-A--L and given the same monoclonal antibody showed only slight decreases in their antibody titers when compared to the titers of (T,G)-A--L-immunized mice treated with NMS. The mice in this experiment received two injections of 100 μg i.p. of aqueous antigen on day 1 and day 8 and 0.4 to 0.5 ml of monoclonal antibody or NMS on days 0, 2, 7, and 9 (a total of 1.9 ml NMS or H10-3.6 per mouse).

Use of a lower dose of the anti-I-A^k monoclonal antibody (a total of 0.15 ml per mouse given in 0.01-ml aliquots 5 times/wk beginning 1 wk before initial antigen injection) had a statistically significant ($P < 0.05$) but much less dramatic effect on the (H,G)-A--L responses without a significant reduction in (T,G)-A--L responsiveness

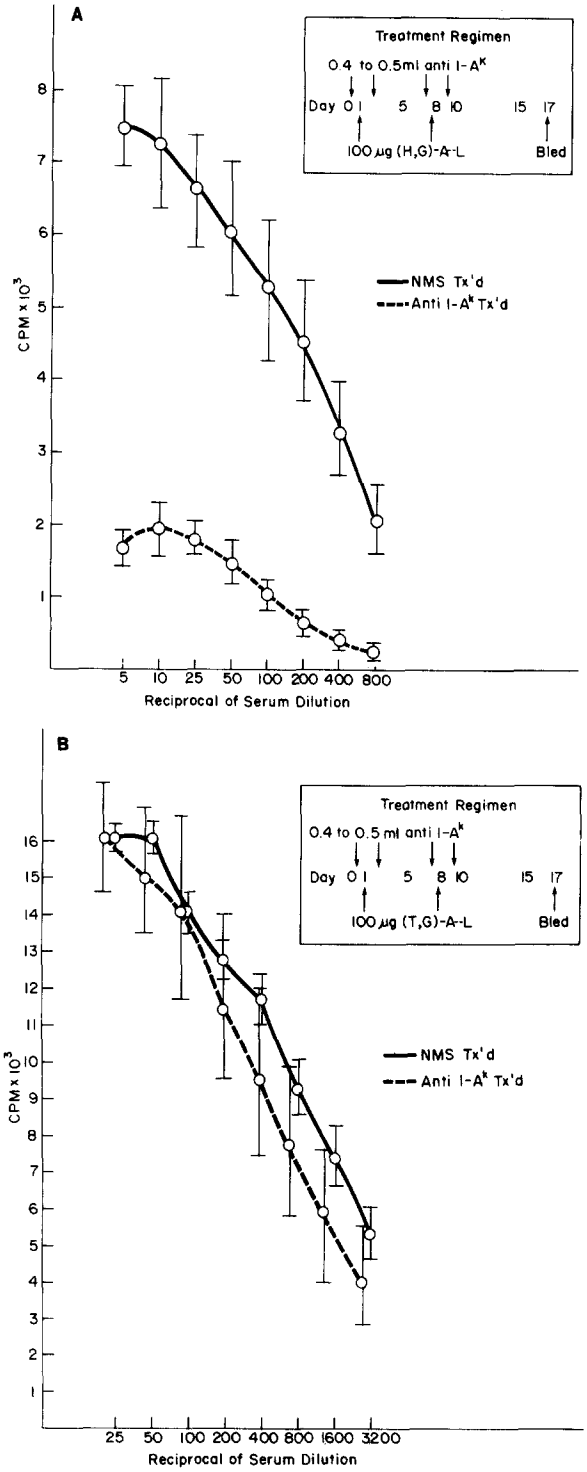

FIG. 1. (H,G)-A--L binding (part A) and (T,G)-A--L binding (part B) of sera from (C3H × CWB)F_1 mice immunized with aqueous antigen and treated with anti-I-A^k in vivo. Data represent means ± SE for binding of sera from groups of four to five female mice tested individually. In part A, $P \leq 0.002$ for all dilutions tested, H10-3.6 vs. controls. In part B, $P \geq 0.18$ for all dilutions tested, H10-3.6-treated vs. controls.

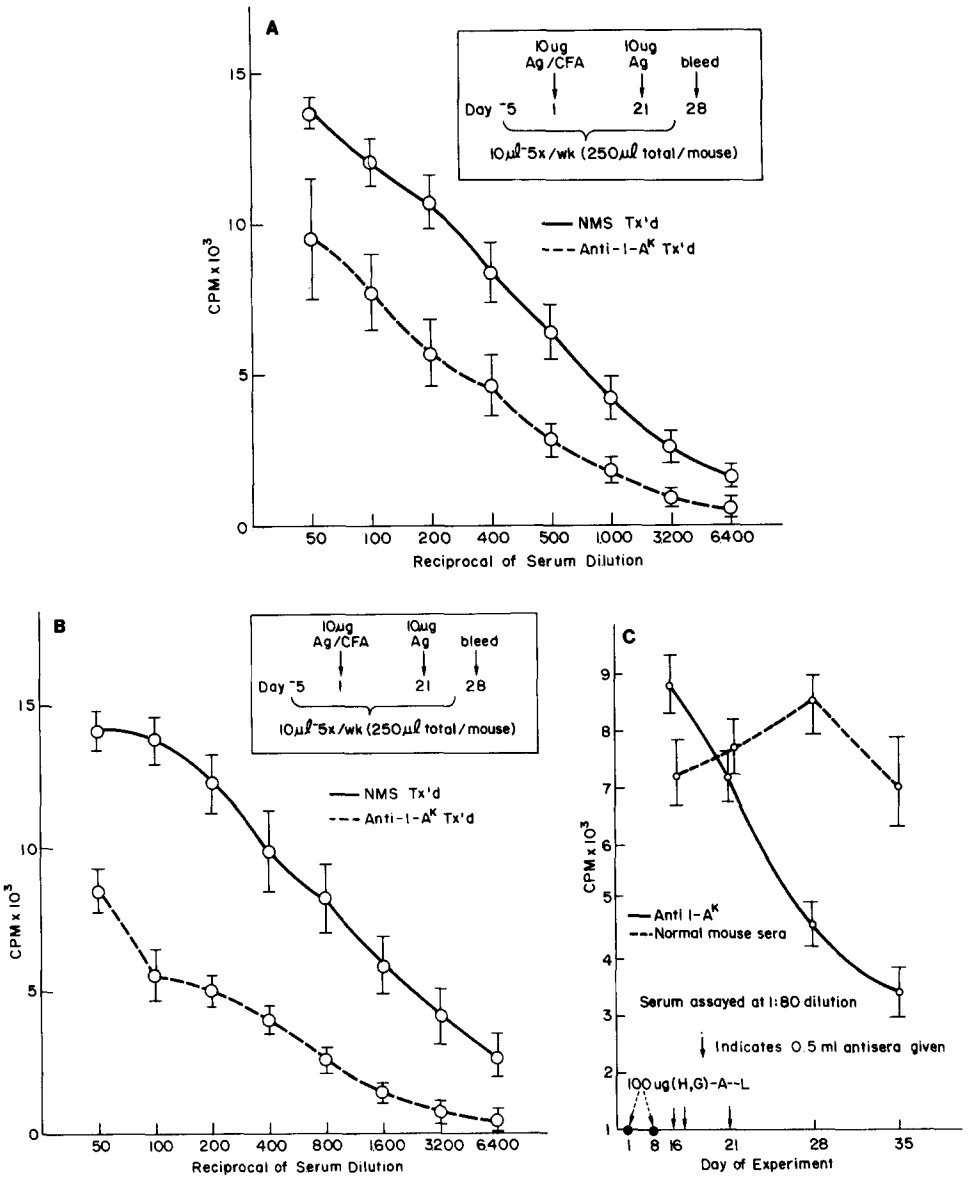

FIG. 2. (H,G)-A--L binding (part A) or (T,G)-A--L binding (part B) of sera from (C3H × CWB)F_1 mice immunized with antigen in adjuvant and treated with anti-I-A^k in vivo. Data represent means ± SE for binding of sera from groups of four to five male mice tested individually. In part A, $P \leq 0.02$ for all dilutions tested, except 1:50, for which $P = 0.033$. In part B, $P \leq 0.01$ for all dilutions tested. Part C shows the effect of anti-I-A^k in vivo on established immune response to (H,G)-A--L in (C3H × CWB)F_1 mice. Data represent means ± SE for sera from four to five male mice tested individually. For days 28 and 35, $P \leq 0.01$, anti-I-A^k-treated vs. controls.

(data not shown). These data demonstrate that the anti-I-A^k monoclonal antibody can induce haplotype-specific suppression of antibody response to an antigen controlled by an *Ir* gene in the *I-A* subregion and that the monoclonal antibody's effect is dose dependent.

Interestingly, the specificity of the anti-I-A treatment was lost when the synthetic polypeptides were injected in CFA. In addition, the daily monoclonal antibody dosage required to induce suppression with CFA immunization, 0.25 ml over a 5-wk period, is considerably less than the dose of monoclonal antibody required for comparable effects with an aqueous immunization regimen.

To test whether the anti-I-A^k monoclonal antibody was effective only when given before immunization, mice were given two aqueous injections of (H,G)-A--L intraperitoneally on day 1 and day 8, bled 15 d after the first immunization, and then given 1.4 ml of anti-I-A^k ascites or NMS in three injections over the next week. Mice treated with the monoclonal antibody had a steady decline in the antigen-binding capacity of their sera after day 16, whereas mice treated with NMS maintained a relatively constant antibody titer. These data demonstrate that anti-I-A^k monoclonal antibody in vivo is capable of suppressing not only primary responses but can also suppress established secondary responses.

Discussion

This study demonstrates that in vivo administration of a monoclonal antibody that recognizes a specific I-A haplotype in a heterozygous F_1 mouse can exert a potent, relatively specific suppression of a humoral response controlled by an *Ir* gene in the *I-A* subregion. However, specificity of the suppression is not obtained in all circumstances, e.g., when the antigen is administered in CFA. Furthermore, these data show that the anti-I-A^k monoclonal antibody can also partially suppress an ongoing, secondary, antibody response.

Previous work with antisera against *I*-region gene products has established their potential in modifying the immune system in vivo. Davies and Staines (15), for example, have shown prolongation of allograft survival with alloantisera directed against the *I*-region products of the donor. Anti-I-J sera in microliter quantities in vivo can modulate immune responses to tumors (16, 17) and to schistosomes (18). An alloantiserum against I-A^k is capable of reducing T cell responses to azobenzenearsonate and to a methylcholanthrene-induced tumor (19, 20). Perry and her colleagues (19) demonstrated that suppression of footpad swelling in a mouse heterozygous for H-2 determinants could be haplotype specific when the antigen is coupled to a parental splenocyte. Sprent (21) demonstrated that the same monoclonal antibody that we used could block the induction of helper T cells when given in vivo. Previous studies have not examined humoral immune responses or demonstrated haplotype specificity in mice heterozygous for H-2 determinants using an antigen under *Ir* gene control.

The mechanism underlying the immunosuppression induced by anti-I-A treatment is currently under investigation. The antibody could potentially exert a cytotoxic effect on cells expressing I-A, interfere with the function of cells expressing I-A, bind or inhibit a soluble factor(s) with I-A determinants, or stimulate suppressor cells or factors.

Although the monoclonal antibody used is an IgG_{2a} and is complement fixing, cytotoxicity is an unlikely mechanism because heterozygous B cells, macrophages, and possibly activated T cells would express both I-A alleles, and then the therapy could not be haplotype specific. Although cytotoxicity could explain the results observed when CFA was used, this explanation again seems unlikely because these animals

suffered no obvious ill effects of the therapy. They appeared vigorous, and the weights of lymphoid organs from treated and control animals did not differ (data not shown).

It is more difficult to decide between the remaining three possibilities: haplotype-specific interference of an I-A-determined cellular function, inhibition of a soluble factor, or stimulation of suppressor cells. If the first possibility is the principle mechanism of action, the data of Perry and colleagues (19, 20) would suggest that the target cell is not solely the B cell because these investigators demonstrate that in vivo anti-I-A alloantisera reduce T cell responses as well. A likely target would be an antigen-presenting macrophage, although the ability of anti-I-A to affect an established immune response might suggest that the mechanism of action does not solely involve antigen presentation.

On the basis of these data, it is difficult to exclude an effect on either a soluble factor(s) or on induction of suppressor cells. This latter possibility has been suggested by several investigators (11, 20, 27–29). These studies include the demonstration that an anti-DR framework antibody induces suppression in vitro (27), that antibodies to immune response gene products induce macrophage suppression of T cell proliferation to myoglobin (11), and that anti-I-A in vivo induces T cell suppressors of footpad swelling after challenge with killed tumor cells (20). Studies to assess the induction of suppression in our system are in progress.

Reconciling the specificity of the effect after aqueous immunization with the nonspecificity after CFA immunization is difficult. This specificity is only relative; possibly higher doses of anti-I-A or greater numbers of animals might have revealed a statistically significant reduction in response to (T,G)-A--L after aqueous immunization. However, unlike the effect seen after immunization with antigen in CFA, the effect of anti-I-A^k is relatively much greater for an antigen that is injected in aqueous solution and is controlled by the I-A haplotype recognized by the monoclonal antibody. The conflict is not due to an artifact of the assay system because results with a Farr assay confirm the solid-phase radioimmunoassay results (data not shown). Interestingly, other investigators have noted that an anti-Ia reagent can be immunosuppressive under some circumstances even if the antigenic response is not under *Ir* gene control (12, 30) or if the anti-I-A recognizes a nonresponder haplotype in an F_1 heterozygous for *Ir* genes (11). In Berzofsky and Richman's system, using antisera against immune response gene products to induce suppression of T cell proliferation (11), the ability to induce suppression depended on the presence of mycobacteria in the adjuvant used to stimulate T cells in vivo. Because antigenic stimulation has recently been reported to increase expression of *I-A* on splenic macrophages (31), one speculation is that CFA immunization makes macrophages more susceptible to the effects of an anti-I-A antibody.

The relative specificity of anti-I-A treatment suggests that ultimately it might provide a useful means of immunotherapy in a variety of human diseases for which an immune response gene might be causally related to disease development, and for which immunotherapy is currently used but is limited by the attendant risks of nonspecificity. Because DR antigens are analogous to Ia antigens (32) and because disease susceptibility associated with DR antigens is a dominant trait with the vast majority of individuals with a given disease heterozygous for the susceptibility gene (33), immunotherapy with anti-DR may ultimately prove feasible. However, the practicality of such therapy depends on toxicity, the availability of haplotype-specific

human anti-DR antibodies, and the role that immune response gene products actually play in sustaining a disease process. The mice appear to tolerate anti-I-A treatment well, especially as judged by their ability to make an immune response to an antigen not under the control of the haplotype to which the antisera is directed; however, more thorough investigation of toxicity is needed. In doses of up to 2 ml over a 10-d period, mice appeared to be vigorous. No evidence of immune complex disease has developed as evidenced by studies of renal histology at autopsy. Lower doses of anti-I-A did not produce a consistent loss in body weight. In more than 100 mice treated with anti-I-A in doses ranging from 0.15–2.0 ml of ascites fluid over a several week period, we observed only two unexpected deaths. In one case, an interstitial pneumonia of unknown etiology developed, and in another, autopsy failed to reveal the cause of death. Clearly, toxicity is a critical, unanswered question in these studies.

Although the eventual applicability of these preliminary studies to human disease will require much more study of both the mechanisms and the risks involved with these antibodies, our preliminary work suggests that in vivo administration of allo-antisera or monoclonal antibodies to *I*-region products can prevent experimental allergic encephalitis in the mouse, susceptibility to which is under control of the MHC (Steinman, L., J. Rosenbaum, R. Sriram, and H. McDevitt, in press).

Summary

Immune response (*Ir*) gene products control immunologic function at several critical sites. We administered in vivo a monoclonal antibody reactive with I-A^k to F_1 mice with the genotype H-$2^{k/b}$. These treated mice made a markedly reduced antibody response to antigen (H,G)-A--L, under the control of I-A^k, but not to antigen (T,G)-A--L, under the control of I-A^b. This relative specificity was lost if the antigen was given in complete Freund's adjuvant rather than aqueous solution. The monoclonal antibody reduced the antibody titer in an ongoing, secondary response as well. Several potential mechanisms can be postulated for this effect. This haplotypic specificity might ultimately be relevant to human disease.

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