CONVERSION OF IMMUNITY TO SUPPRESSION
BY IN VIVO ADMINISTRATION OF I-A
SUBREGION-SPECIFIC ANTIBODIES*

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Cell surface glycoproteins encoded by the I region of the murine major histocompatibility complex play a central role in regulating lymphoid cell interactions. Macrophage-T cell (1, 2) and T cell-B cell (3, 4) collaborations are dependent upon homology at this region, in most cases mapping to the I-A subregion of the H-2 complex. Because it has not been possible to separate by recombination I region-associated (Ia) antigen expression and immune response (Ir) gene phenotypes of inbred mouse strains, it appears that Ia antigens may be the vehicles of Ir gene function (5). This is supported by biochemical and functional studies on the genetic control of Ia antigen synthesis (6, 7). T cells (8), B cells (9, 10), and macrophages (11, 12) have all been implicated as possible sites of Ir gene expression, although the precise mechanism(s) of gene action are still a matter of conjecture (13).

Immune responsiveness to antigens under Ir gene control has been modulated experimentally through the use of antibodies specific for the cell surface products of these loci. Inhibition of antigen-specific T cell proliferation in vitro has been accomplished using monoclonal anti-Ia antibodies specific for the controlling Ia subregion (14), or, in the case of antigens under complementing Ir gene control, with antibodies raised against the hybrid Ia determinant (5). Such antibodies act at the level of the macrophage under these conditions (15) and function, at a minimum, by blocking T cell binding to I-A$^+$ antigen-presenting cells (16). The observable effect of these manipulations is the abrogation of T cell stimulation because of ineffective macrophage-T cell interaction.

I subregion-specific antibodies have also been shown to exert profound effects on antigen-specific reactivity when administered in vivo, where results are again consistent with antibody-mediated interference with macrophage-dependent T cell antigen recognition. Previous work from this laboratory has revealed that Lyt-1$^+$ T cell responses to syngeneic tumor antigen, assayed either by tumor rejection (17) or by the expression of delayed type-hypersensitivity (DTH)[1] (18), are inhibited by the intravenous injection of anti-I-A alloantisera. Inhibition of DTH in a hapten model was shown to be specific for the I-A genotype of the hapten-coupled antigen-presenting

[1] *Abbreviations used in this paper:* C', complement; CY, cyclophosphamide; DTH, delayed-type hypersensitivity; HBSS, Hanks' balanced salt solution; MCA, methylcholanthrene; Te, effector T cell; Ts, suppressor T cell.

cell (18), and resulted in a loss of transferrable DTH reactivity. Other laboratories have reported similar effects of in vivo monoclonal anti-I-A antibody treatment on T helper cell activity (19), Ir gene-controlled antibody production (20), and, most recently, on the induction of murine experimental allergic encephalitis (21). The capacity of antibodies directed against products of the Ir gene loci to alter the course of cell-mediated immunity in vivo in each of these widely divergent systems supports the hypothesis that this means of therapy may be effective in regulating T cell responses to any antigen that undergoes macrophage processing before T cell recognition (18).

Because of the complexity of cell interactions that serve to maintain order within the immune system, it would appear almost inevitable that perturbation of any single aspect of a multicellular response may have repercussions at some other level. In this report, we demonstrate this to be true in animals treated with I-A subregion-specific antibodies. As briefly described elsewhere (22), the administration of anti-I-A antibodies to mice immunized for tumor-specific DTH reactivity is associated with the development of antigen-specific suppressor T cell (Ts) activity. It is proposed that activation of the suppressor cell circuit provides a mechanism to maintain $Lyt-1^+$ T cell nonresponsiveness in the absence of continued antibody treatment (18), thus amplifying the primary effect of anti-I-A antibodies on macrophage presentation of antigen.

Materials and Methods

Mice. 8–10-wk-old female A/J ($H-2^a$) mice, obtained from The Jackson Laboratory, Bar Harbor, ME, were used in all experiments.

Immunization. The maintenance and growth characteristics of the S1509a and SA1 A/J methylcholanthrene (MCA)-induced fibrosarcomas have been described previously (23). Animals were immunized by subcutaneous injection of 10^6 ascites-derived S1509a cells given 7,800 rad of irradiation in a Gammacell 40 (Atomic Energy of Canada, Ottawa, Canada) (18). DTH responses were elicited by footpad challenge 5 d later with 10^6 similarly irradiated cells in a 30-μl vol (18). Differences in swelling between injected and uninjected footpads were measured after 24 h and compared with swelling responses of unimmunized control mice. This immunization protocol has been shown to induce transferable, antigen-specific $Lyt-1^+$ T cell-mediated DTH reactivity (24), but differs from a live tumor cell challenge in that the suppressor circuit is not activated (23, 24).

Adoptive Transfers. Adoptive transfers were performed as described previously (18) using single cell suspensions of spleen, thymus, or lymph nodes obtained 5 d after immunization. 5×10^7 cells were injected intravenously into normal recipients immunized simultaneously with irradiated S1509a cells subcutaneously as above.

Cyclophosphamide Treatment. Elimination of suppressor cell precursors was attempted by intraperitoneal injection of 20 mg/kg Cytoxan (Mead Johnson & Co., Evansville, IN) in saline 3 d before immunization (25). This protocol has been effective in reducing suppressor cell activity in mice bearing progressive MCA-induced tumors, resulting in the loss of transferrable suppression (25).

Anti-I-A Antibodies. Antibodies used in most experiments consisted of a (B10 × LP.RIII)F_1 anti-B10.A(4R) antiserum specific for K and I-A antigens of the $H-2^a$ haplotype. Characterization of this antiserum and the singular role of anti-I-A antibodies in inhibition of T cell responses have been reported previously (17). In the present experiments, animals received daily intravenous injections of 2–5 μl antiserum in a 0.2 ml. volume of Hanks' balanced salt solution (HBSS). Monoclonal anti-I-A^k antibodies used in certain experiments consisted of protein A-Sepharose-purified culture supernatants of the 10-2.16 or 10-3.6 hybridomas (both recognizing public specificity Ia.17). The latter antibodies were kindly donated by Dr. R. Germain, Harvard Medical School, Boston, MA. 20 μg of these antibody preparations were administered intrave-

nously per mouse per day in 0.2 ml HBSS. Monoclonal anti-I-A^d antibody used in certain specificity experiments was derived from culture supernatants of the MKD6 hybridoma cell line, generously donated by Dr. J. Bersofsky, National Cancer Institute, Bethesda, Md.

Antibody and Complement (C') Treatment. The murine hybridoma line, HO-13.4, obtained from the Salk Institute, La Jolla, CA, was used as a source of monoclonal anti-Thy-1.2 antibody. Aliquots of 2 × 10^8 spleen cells were resuspended in 4 ml HO-13.4 culture supernate and incubated on ice for 30 min. After washing in HBSS, 2 ml of a 1:12 dilution of low toxicity rabbit C' (Cedarlane Laboratories, Ltd., Hornby, Ontario, Canada) was added, and cells were incubated for an additional 30 min at 37°C. Cells were then washed twice more and subjected to a second treatment by incubation in 1 ml HO-13.4 supernate plus 1 ml of a 1:6 dilution of C' for 15 min at 4°C followed by 5 min at 37°C. Cells remaining after this double treatment were resuspended at 2.5 × 10^8 viable cells/ml in HBSS and 0.2 ml of this suspension was injected intravenously into recipient mice.

Statistical Significance. The mean difference between groups of five mice were analyzed on the Wang programmable computer (Wang Laboratories, Inc., Lowell, MA) using the Student's t test.

Results

Generation of Antigen-specific Suppressor Cells as a Consequence of Anti-I-A Antibody Treatment In Vivo. The expression of Lyt-1$^+$ T cell responses to syngeneic MCA-induced tumor antigen can be inhibited by the in vivo administration of I-A subregion-specific antisera (17, 18), presumably as a result of interference with macrophage-T cell interactions during antigen presentation in vivo (18). Because previous studies revealed that the administration of only 4 μl of an anti-K^k, I-A^k antiserum over a 2-d period was sufficient to inhibit DTH responsiveness 5 d later (18), it was reasoned that some additional mechanism may be functioning to amplify the in vivo activity of these antibodies. As one approach to this issue, experiments were designed to assay for suppressor cell function in anti-I-A antibody-treated mice (22).

The question of Ts activation by anti-I-A antibody administration was addressed by analyzing the DTH response to syngeneic tumor antigen, because immunization for DTH can be achieved using irradiated tumor cells and is not complicated by the spontaneous activation of suppressor cells that occurs after a live tumor challenge (23). A/J (H-2^a) mice were inoculated subcutaneously with 10^6 irradiated S1509a cells and treated daily with 5 μl (B10 × LP.RIII)F$_1$ anti-B10.A(4R), anti-K^k, I-A^k alloantiserum. The in vivo activity of this antiserum has been attributed solely to the contribution of anti-I-A^k alloantibodies (17). After 5 d, spleen cells from these or from untreated control mice were adoptively transferred to normal A/J recipients. Recipients were immunized subcutaneously within the hour with an identical dose of S1509a cells and DTH responses elicited 5 d later by footpad challenge with the immunizing tumor cell. Results of one such experiment are shown in Fig. 1. Animals receiving no spleen cells, or cells from normal or immune (but untreated) donors expressed comparable levels of DTH reactivity. In contrast, animals receiving spleen cells from S1509a-immune, anti-I-A^k antibody-treated donors exhibited a significantly depressed DTH response, indicating the transfer of cells capable of suppressing the induction of recipient Lyt-1$^+$ T cell responses.

The specificity of suppression is demonstrated in Table I. In this experiment, spleen cells from S1509a-immunized anti-I-A^k antibody-treated mice were adoptively transferred to recipients immunized with S1509a or with SA1, a second A/J MCA-induced fibrosarcoma that cross-reacts with S1509a at the effector level but induces a distinct suppressor cell (26). As indicated, suppressor cells induced in animals primed with
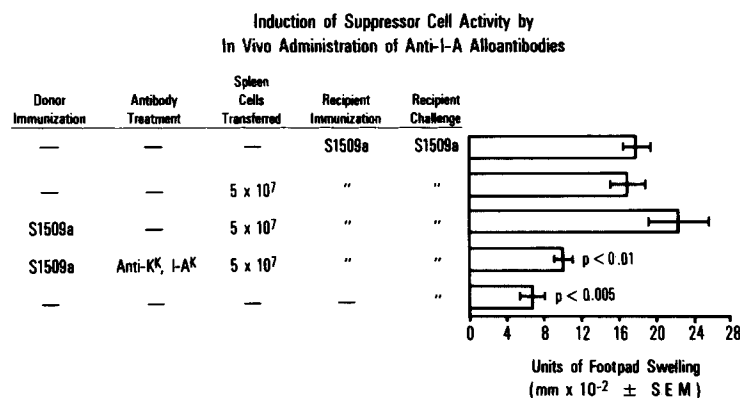
Induction of Suppressor Cell Activity by
In Vivo Administration of Anti-I-A Alloantibodies

Fig. 1.   Induction of suppression by in vivo anti-I-A antibody treatment. A/J mice were immunized by subcutaneous injection of 10^6 8,000-rad-irradiated S1509a cells and treated daily for 5 d with 5 μl (B10 × LP.RIII)F_1 anti-B10.A(4R), anti-K^k, I-A^k alloantiserum. Spleen cells from these or from normal or immunized (but untreated) mice were removed on day 6 and adoptively transferred to syngeneic recipients immunized subcutaneously with an identical dose of irradiated S1509a cells. DTH responses were evoked in recipient mice 5 d later by footpad challenge with 10^6 irradiated S1509a cells, and footpad swelling was measured 24 h after challenge.

TABLE I

Specificity of Anti-I-A Antibody-induced Suppressor T Cells

Donor immunization	Antibody treatment*	Spleen cells transferred	Recipient immunization‡	Recipient challenge‡	Units of footpad swelling
					$mm \times 10^{-2} \pm SEM$
—	—	—	S1509a	S1509a	27.4 ± 2.6
S1509a	Anti-I-A^k	5 × 10^7	S1509a	S1509a	13.5 ± 1.4§
—	—	—	—	S1509a	11.4 ± 1.2§
—	—	—	SA1	SA1	23.1 ± 2.1
S1509a	Anti-I-A^k	5 × 10^7	SA1	SA1	20.6 ± 1.8
—	—	—	—	SA1	11.2 ± 1.0§

* Animals received intravenous injections of 2 μl/d (B10 × LP.RIII)F_1 anti-B10.A(4R), anti-K^k, I-A^k antiserum daily for 5 d.
‡ Immunization was accomplished by subcutaneous injection of 10^6 8,000-rad-irradiated S1509a or SA1 cells in a 100-μl vol. Footpad challenge was performed by the same number of irradiated tumor cells but in a 30-μl vol. Footpad swelling was measured 24 h later.
§ Statistically significant, $P < 0.01$.

S1509a cells specifically inhibited responses to S1509a and not to related SA1 tumor antigens. The specificity of these cells for antigens unique to the S1509a tumor is similar to that of Ts arising during progressive tumor growth in vivo (26), although their activation under these conditions is dependent upon antibody-mediated interference with the normal sequence of cellular events rather than a spontaneous response to replicating tumor cells.

Distribution and Cyclophosphamide Sensitivity of Anti-I-A Antibody-induced Ts.   Studies in a number of laboratories have shown that Ts cells reside primarily in the spleen and thymus (27-29), and that Ts activity in these organs can be ablated by the injection of low doses of cyclophosphamide (CY) before antigen sensitization (25, 30). The distribution and CY sensitivity of suppressor cells induced by anti-I-A antibody treatment were investigated to determine the extent of similarity between Ts active in these widely divergent systems. The suppressive capacity of spleen, thymus, and

lymph node cells from anti-I-A antibody-treated mice is demonstrated in Fig. 2. It is apparent that suppression, shown to be T cell-dependent by its sensitivity to treatment with monoclonal anti-Thy-1.2 and C', can be adoptively transferred with splenocytes or thymocytes but not with lymph node cells. A similar distribution has been identified for Ts induced during tumor progression in this system, although lymph nodes also appeared to contain a small proportion of the relevant cells (29). The generation of Ts by a 2-d regimen of antibody treatment is also depicted in Fig. 2, indicating that the cellular events relevant to Ts activation occur during the first 2 d of sensitization, corresponding to the expected period of macrophage presentation of tumor antigen to Lyt-1$^+$ T effector cells (Te). These results suggest that the induction of Ts activity may be a primary means of maintaining inhibition of Te responses in the absence of further antibody treatment.

Based upon these findings, it became of interest to determine the extent to which suppressor cell function contributed to the in vivo activity of anti-I-A antibodies. To accomplish this, we attempted to ablate Ts precursors by intraperitoneal injection of 20 mg/kg CY 3 d before immunization, a protocol that has been shown effective in diminishing Ts activity in mice given a live tumor challenge (25). Results presented in Table II demonstrate the effect of CY pretreatment on the expression of DTH in anti-I-A antibody-treated mice. Animals treated with anti-I-A antiserum alone exhibited suppressed DTH reactivity, as expected, whereas injection of CY alone caused a slight enhancement of T cell responses. The combination of antibody plus CY treatment, however, reversed the suppression normally obtained by antibody administration, indicating that the full effects of antibody treatment may be observed only in the presence of an intact suppressor cell system. Interference with macrophage-T cell communication alone by the small concentration of antibody used in these

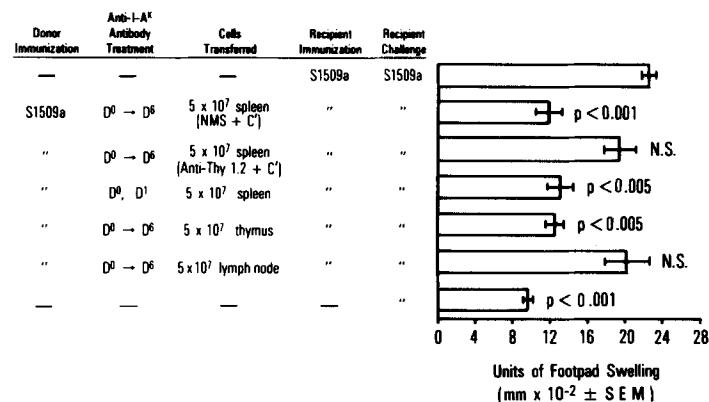Distribution of Anti-I-A Antibody-Induced Suppressor T Cells

Fig. 2. Distribution of anti-I-A antibody-induced Ts. A/J mice, immunized on day 0 with 10^6 8,000-rad-irradiated S1509a cells, were treated with 5 μl/d (B10 × LP.RIII)F$_1$ anti-B10.A(4R) anti-K^k, I-A^k antiserum on the days indicated. Cells were removed on day 6 and adoptively transferred without further treatment or after treatment with normal mouse serum or anti-Thy-1.2 plus C'. Recipients were immunized simultaneously by subcutaneous injection of 10^6 irradiated S1509a cells and challenged with an identical dose of cells in the footpad 5 d later. Swelling responses were measured 24 h after challenge.

TABLE II

CY Sensitivity of Anti-I-A Antibody-induced Ts

CY pretreatment	Immuniza-tion*	Antibody treatment‡	Challenge	Units of footpad swelling
				$mm \times 10^{-2} \pm SEM$
—	S1509a	—	S1509a	26.5 ± 3.4
—	S1509a	Anti-I-A^k	S1509a	14.8 ± 2.2§
20 mg/kg	S1509a	—	S1509a	28.0 ± 3.2
20 mg/kg	S1509a	Anti-I-A^k	S1509a	24.5 ± 3.4
—	—	—	—	8.5 ± 2.3§

* See footnote to Table I.
‡ See footnote to Table I.
§ Statistically significant, $P < 0.02$.

TABLE III

Inhibition of Tumor-specific DTH with Monoclonal Anti-I-A Antibody In Vivo

Immunization	Anti-I-A anti-body treatment*	Challenge	Units of footpad swelling
			$mm \times 10^{-2} \pm SEM$
S1509a	—	S1509a	22.6 ± 0.9
S1509a	serum	S1509a	10.2 ± 1.3‡
S1509a	10-2.16	S1509a	14.2 ± 1.5‡
S1509a	10-3.6	S1509a	13.3 ± 2.1‡
—	—	S1509a	8.9 ± 1.2‡

* Animals received 2 μl/d (B10 × LP.RIII)F$_1$ anti-B10.A(4R), anti-K^k, I-A^k alloantiserum or 20 μg/d purified 10-2.16 or 10-3.6 hybridoma anti-I-A^k antibody for 5 d.
‡ Statistically significant, $P < 0.005$.

experiments appears to be insufficient to significantly dampen the expression of Lyt-1$^+$ T cell-mediated immunity.

Inhibition of DTH with Monoclonal Anti-I-A Antibodies. Because of the potential specificity problems associated with the use of a polyvalent alloantiserum, attempts were made to reproduce the inhibitory effects of anti-I-A^k alloantibodies by the administration of monoclonal anti-I-A^k antibody reagents. A/J mice were immunized by the usual protocol by subcutaneous injection of 10^6 irradiated S1509a cells and injected intravenously with 2 μl/d (B10 × LP.RIII)F$_1$ anti-B10.A(4R), anti-K^k, I-A^k alloantibodies or with 20 μg/d of protein A-Sepharose-purified anti-I-A^k hybridoma culture fluid. Hybridomas used in these experiments consisted of the 10-2.16 and 10-3.6 cell lines that secrete monoclonal antibodies recognizing an I-A determinant corresponding with public specificity Ia.17 (31). As indicated in Table III, the administration of either of these monoclonal antibody reagents inhibited the DTH response elicited upon footpad challenge with 10^6 irradiated S1509a cells. Inhibition was dose-dependent (data not shown) and was achieved only by the administration of antibodies recognizing the relevant I-A determinants, as treatment with similar quantities of a monoclonal anti-I-A^d reagent had no effect on DTH responsiveness (data not shown). These results suggest that the cellular events involved in Lyt-1$^+$ T

cell activation in vivo can be dramatically altered by blocking single determinants on the antigen-presenting cell surface.

Discussion

Manipulation of the cellular immune response by the in vivo administration of anti-I-A antibodies has now been accomplished in several experimental systems (17–21, 32). Results in each of these studies can be interpreted to reflect an effect of antibody on the induction of Lyt-1$^+$ T cell immunity, observed as decreased DTH (18), helper activity (19, 20), or autoimmune (21) responsiveness. We now report that one of the ultimate effects of anti-I-A antibody administration in vivo is the induction of antigen-specific Ts activity.

We have been involved in an analysis of the effects of in vivo anti-I-A antibody treatment on the DTH and tumor rejection response of A/J mice to a syngeneic MCA-induced fibrosarcoma, S1509a (17, 18). Each of these responses is mediated by I-A(−) T cells expressing the Lyt-1$^+$ phenotype (22, 24), and can be inhibited by the intravenous administration of an anti-I-A^k alloantiserum (17, 18). Several lines of evidence suggested a primary effect of antibody on the initiation of Lyt-1$^+$ T cell responses by interference with I-A-restricted antigen presentation, although it was difficult to reconcile how such minute quantities of antibody could exert these potent biological effects, particularly in view of the absorptive capacity of Ia$^+$ B cells present in the circulation during intravenous antibody administration. The present studies provide one viable explanation for this dilemma, because it appears that maintenance of the depressed state of immunity is mediated by Ts.

Suppression was evidenced by the capacity of spleen or thymus cells from antibody-treated mice to abrogate the induction of recipient DTH responses to tumor antigen. Cells capable of transferring this suppression were shown to be T cells by their sensitivity to monoclonal anti-Thy-1.2 plus C'. Specificity of Ts for S1509a tumor antigen(s) was demonstrated by the failure of these cells to alter responses of recipients immunized with SA1 tumor cells, a second MCA-induced fibrosarcoma (26). In these respects, i.e., distribution, action during the induction phase of the DTH response, and antigen specificity, the anti-I-A antibody-induced Ts closely resembles Ts activated spontaneously during the course of progressive tumor growth (26, 29). The mere presence of tumor antigen as it is expressed on the surface of irradiated S1509a cells is an insufficient stimulus for Ts generation, however, as shown by the transfer of spleen cells from immunized but untreated donors. Suppressor cell induction under these conditions is dependent instead upon an undetermined sequence of cellular events initiated by the administration of I-A subregion-specific antibodies.

The absolute requirement for suppressor cell function in this system was shown by demonstrating that pretreatment with CY to eliminate Ts precursors (25) also abrogated the inhibitory effects of in vivo antibody administration. These findings suggest that the anti-I-A antibody-induced Ts is (a) CY sensitive, and (b) a necessary participant in the cascade of events that occurs after antibody administration. Although suppressor activity has not yet been reported in other in vivo models of anti-I-A antibody effects (19–21, 32), several examples exist demonstrating supression after in vitro anti-I-A antibody treatments (33–35). Most notably, Broder et al. (33) have identified in cultures of mitogen-stimulated human lymphocytes upon addition of an antiserum to human Ia-like antigens (p 23, 30). More recently, Bersofsky and

Richman (35) have described transferrable suppression with washed, anti-I-A anti-body-treated macrophages, although it was not determined whether the macrophage suppressed secondary cultures directly or by inducing a Ts subset. It will be of great interest to compare the results we have obtained with the in vivo models of Sprent (19), Rosenbaum et al. (20), and Steinman et al. (21), where inhibition has been observed by similar antibody treatments.

Several mechanisms can be envisioned to account for the generation of suppressor T cells in the presence of antibodies that block macrophage I-A molecules. It is considered unlikely that suppression is the result of a direct interaction between antibody molecules and receptors on the Ts cell surface, because Ts reactive against tumor (36) or numerous other (13) antigens have been shown to express I-J- or I-C-encoded determinants, but consistently lack I-A-encoded cell surface structures. Thus, there is no evidence for a target of anti-I-A antibody on a T cell that effects suppression. Experiments performed in vitro have demonstrated the induction of suppressor cells by high concentrations of soluble antigen (37) or by depletion of macrophages and other adherent cells (38). There is no *a priori* reason to assume a direct effect of anti-I-A antibody treatment on antigen levels because ingestion and catabolism of antigen by macrophages proceeds uninhibited under these conditions (39). The presence of such antibodies may alter antigen clearance indirectly, however, by limiting T cell recruitment of mononuclear cells involved in antigen handling, thereby creating a condition of antigen excess. Alternatively, I-A-specific antibodies may induce suppression by local effects on the redistribution of proteins within the macrophage plasma membrane. Although little information is available concerning the membrane organizational events underlying I-A-restricted T cell antigen recognition, there is evidence that I region gene products function at the antigen-presenting cell surface (11) at some point after antigen ingestion and catabolism (39). If I-A molecules serve as receptors for fragments of internally processed antigen (39), the addition of antibodies capable of blocking membrane antigen-I-A interactions may allow the expression of processed antigen in an uncomplexed form or perhaps in association with macrophage I-J determinants (40). Antigen presented in this context may then provide an activation signal for a suppressor cell precursor. Indeed, evidence has been presented for a role of macrophages in suppressor cell interactions (41, 42), although the point at which antigen presentation may be required has not been determined. Further functional and phenotypic characterization of the anti-I-A antibody-induced Ts as a Ts₁, Ts₂, or Ts₃ cell, each of which has distinct receptors specificities and activation signals (43–46), should aid in elucidating the mechanism by which Ir gene function is blocked and Ts induction occurs under these conditions.

The observation that antigen-specific suppressor T cells are induced by in vivo treatment with I-A subregion-specific antibodies serves not only to clarify the biological actions of these proteins, but also provides a means for generating functional suppressor molecules against the myriad of antigens that are dependent upon macrophage I-A-restricted presentation. The potential benefits of this approach to clinical manipulation of inappropriate T cell responses has already been demonstrated by the capacity of anti-I-A antibody treatment to prevent the development of murine experimental allergic encephalitis (21). Based upon our previous observation that I-A-specific antibodies also inhibit host T cell responses to non-H-2 histocompatibility antigens (24), we are currently focusing on anti-I-A treatment as a therapeutic

approach to manipulating transplant rejection in animals differing at minor histocompatibility loci. The immunological specificity inherent in this means of treatment, as well as its demonstrated effectiveness in regulating cellular responses in a variety of experimental situations, suggests that similar reagents may be readily applicable to the modulation of Ir gene function in human disease.

Summary

The in vivo administration of antibodies specific for gene products of the I-A subregion represents an immunologically specific approach to the manipulation of Ly-1$^+$ T cell responses to antigen. This has been demonstrated previously by the capacity of anti-I-A antibody treatment to abrogate T cell-mediated delayed-type hypersensitivity (DTH) responses to syngeneic tumor antigen, hapten, and non-H-2 histocompatibility antigens. Evidence obtained in these studies suggested that the primary action of antibody was related to its ability to interfere with macrophage-T cell interactions during antigen presentation, consistent with the demonstration that similar antibodies inhibit T cell binding to antigen-pulsed macrophages in vitro. Results presented in this report provide evidence for an additional consequence of in vivo antibody administration that may be secondary to any direct effects on I-A-restricted antigen presentation. Thus, animals treated with I-A subregion-specific antibodies also develop a population of antigen-specific suppressor T cells (Ts) capable of inhibiting recipient Ly-1$^+$ T cell responses to tumor antigen. The induction of suppression appeared to be an essential component of the total biological activity of these antibodies, because elimination of Ts precursors by cyclophosphamide also abrogated the antibody-mediated inhibition of DTH responsiveness. These results are discussed with respect to the possible mechanisms of Ts activation by anti-I-A antibody administration, and the general applicability of this approach as a means of clinical immunotherapy to limit inappropriate T cell responses in human disease.

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