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BRIEF COMMUNICATIONS

EVIDENCE THAT INITIAL T CELL DEPLETION SUPPRESSES ANTIANTIBODY FORMATION AND PRESERVES IMMUNOSUPPRESSION AFTER POLYCLONAL ATG[1]

It has been well established that the injection of heterologous monoclonal antibodies (mAb) elicits antiantibodies in patients (*1–6*). In mice anti–T cell mAb induces antibodies that react with the variable and constant regions of the injected mAb (*7*). Accelerated antibody clearance resulting in inhibition of immunosuppression ensues (*8*). Cell-binding antibodies such as anti–T cell mAb induce a particularly strong and in escapable antiantibody response. In mice, for instance, no tolerance of rat anti–T cell mAb could be obtained by preinjecting deaggregated normal rat Ig or anti-L3T4 (CD4) mAb, methods of tolerance induction that were successful with polyclonal rat IgG or non–cell-binding rat mAb IgG (*7*). Whether tolerance to the cell antigen-binding site (i.e., idiotype) can be attained after mAb have been "humanized" by molecular biological methods is presently under investigation (*9*).

The findings with anti–T cell mAb are partly in keeping with early studies on the sensitizing effect of polyclonal heterologous antilymphocyte globulin (ALG) (*10–15*). As with rat anti–T cell mAb, rabbit or horse ALG proved more immunogeneic than its normal non–cell-binding heterologous Ig control. For instance, mice injected with 200 μg ALG were found to be at least eight times more responsive than mice injected with the same amount of normal rabbit Ig when tested for immune elimination of subsequently injected radioiodinated Ig (*10*). The paradox that immunosuppressive heterologous anti–T cell antibody Ig is more immunogenic than normal Ig was explained by the presentation of the antigen in a "particulate" form, i.e. on cells. Examples were cited where heterologous antigens such as thyroglobulin adsorbed on acrylic resin particles or bentonite-adsorbed ovalbumin were more immunogenic than when injected in a soluble form (*13*). Less clear was the anti-immunosuppressive consequence of antibody formation to ALG. No significant shortening of ALG-induced prolongation of skin allograft survival was observed in mice that had been presensitized to rabbit ALG and showed immune elimination of radioiodinated rabbit Ig (*14*). In another report, skingraft survival in mice treated with ALG was shortened—though not dramatically—following a sensitizing dose of ALG (*16*). The specificity of antiantibodies against ALS must also be considered. In one report several mice developed antibodies against the β globulin component of rabbit globulin. In that case the immunosuppressive effect was not diminished (*17*).

The question as to why ALG-induced T lymphocytopenia does not counteract the formation of antiantibodies to ALG, also remains unresolved. A more general question remains, regarding the importance of T cells in sensitization against ATG and whether antibodies to B cells present both in ALG and ATG contribute directly to antiantibody formation, for instance by triggering B cells that express Ig receptors specific for epitopes on heterologous Ig.

We have now demonstrated that T lymphocytes in fact initiate sensitization to ATG, resulting in a complete blockage of its immunosuppressive effect. The decisive role of T cells in antiantibody formation to ALG, which is not apparent in mice rendered T-lymphopenic by heterologous ALG, became evident in a mouse model in which murine antibodies induced T cell depletion. We show that in mice, T lymphopenia induced by murine anti–Thy-1 mAb suppresses subsequent sensitization to ALG. Our observation that T lymphopenia induced by autologous anti–Thy-1 mAb can suppress the immune response to polyclonal heterologous antibodies may lead to an understanding of the mechanism of sensitization to cell-binding antibodies and indicate methods for circumventing antiantibody formation.

ATG was raised in rabbits by the injection of mouse thymocytes as previously described (*18*). IgG fractions obtained by ammonium sulfate precipitation and DEAE column chromatography were adjusted to a concentration of 10 mg IgG per ml. Sensitization to ATG was obtained by four weekly injections of 0.25 ml ATG i.p. into CBA or C57BL/6 mice (9–14 week old), bred in our institution from stock obtained from the Jackson Laboratory. For bone marrow transplantation, irradiated (C57BL/6×CBA)F1 recipients and C57BL/ 6 donors were used. Mouse anti–Thy-1.2 mAb (MmT1) of IgG2a subclass was derived according to the procedure of Köhler and Milstein (*19*). Spleen cells of AKR/J mice immunized 4 times at 3-week intervals with thymocytes of AKR/Cum were fused with the myeloma cell line PO3×63-AG 8.653 (ATCC: CRL 1580). To suppress antiantibody formation, mice were injected i.p. with 1 mg of this mAb on days −6 and −3 before inducing sensitization to ATG. Splenic T cells were reduced to 3% on day −1 (Antica M, unpublished observation).

First we studied the occurrence of mouse antibodies to ATG in vitro with a cell-binding inhibition test. Sera of 4 sensitized mice were titrated against diluted ATG (5 μg Ig) in the presence of 2×10^5 mouse thymocytes. They were compared with those of mice receiving anti–Thy-1.2 mAb before sensitization. After 30 min incubation at room temperature the cells were washed three times. Binding of rabbit ATG to thymocytes and its inhibition of binding due to serum with anti-ATG antibodies of sensitized mice was tested in a solid-phase ELISA with peroxidase-labeled (POX) goat anti-rabbit Ig (Tago, Medac, Hamburg, FRG). Binding to thymocytes of ATG complexed with mouse anti-ATG antibodies was tested with POX goat anti-mouse Ig (Sigma, Deisenhofen FRG). After 0.1 ml substrate solution (Fluka 78440, Neu Ulm, FRG) had been added, absorbance was determined at 405 nm.

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The results are shown in Figure 1. Sera of sensitized mice inhibit binding of ATG to cells completely. At higher dilution, where there is partial inhibition, mouse antibodies can be detected being complexed to cell-bound ATG. Two injections of anti–Thy-1.2 mAb suppress the induction of inhibitory antiantibodies in mice treated with ATG.

The in vivo consequences of antiantibody formation and its suppression by anti–Thy-1.2 mAb were studied in a skin allograft and a graft-versus-host model (Fig. 2). CBA mice tolerated fully H-2–incompatible C57BL/6 skin grafts as long as they were treated with ATG 3 times a week. The skin grafts were rejected within 2–3 weeks after discontinuation of ATG on day 40 posttransplant. In contrast, CBA mice sensitized to ATG showed little prolongation of skin graft survival in spite of continuous injections of ATG as applied above. The sensitizing effect of ATG injections was suppressed by two injections of anti–Thy-1 mAb, so that tolerance to skin allografts under continuous ATG treatment was obtained (Fig. 2A). GVHD mortality was suppressed in (C57BL/6×CBA)F1 mice irradiated with 9 Gy (104 rad/min) and transfused with 2×10^7 bone marrow together with 5×10^7 spleen cells from C57BL/6 mice injected with 0.25 ml ATG 6 days prior to transplantation. If the donors had been presensitized, immunosuppression was blocked and 100% GVHD mortality occurred within 4 weeks. Again, anti–Thy-1 mAb suppressed sensitization, thus allowing ATG to suppress GVHD (Fig. 2B). Control mice treated with anti–Thy-1 mAb, but without subsequent sensitization and/or treatment with ATG, showed no immunosuppression when tested in the skin allograft or GVHD models 4 weeks later.

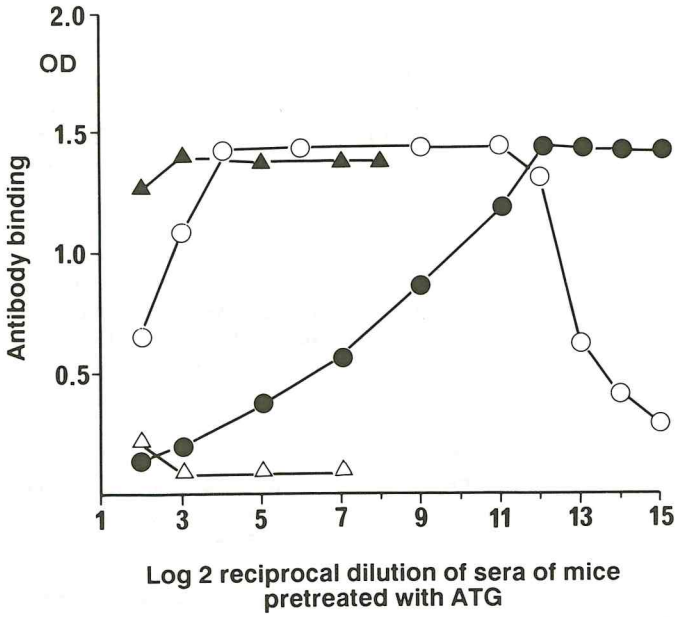

FIGURE 1. Suppression of antiantibody-induced inhibition of binding of ATG to thymocytes (EIA). Sera of mice sensitized to rabbit ATG (●,○) or non (▲,△) sensitized (because of pretreatment with anti–Thy-1.2 mAb) were incubated with rabbit ATG and mouse thymocytes. POX-labeled goat anti-rabbit Ig was added. This allowed testing of the inhibition of ATG binding to thymocytes (due to anti-ATG antibodies in sera of sensitized mice) (●) and of the suppression of such inhibitory antibodies due to pretreatment with anti–Thy-1.2 mAb (▲). Addition of POX-labeled goat anti-mouse IgG was added in order to test binding to thymocytes of mouse anti-ATG antibodies complexed with ATG (○) or absence of such complexes (due to suppression of sensitization by pretreatment with anti–Thy-1.2 mAb [△]).

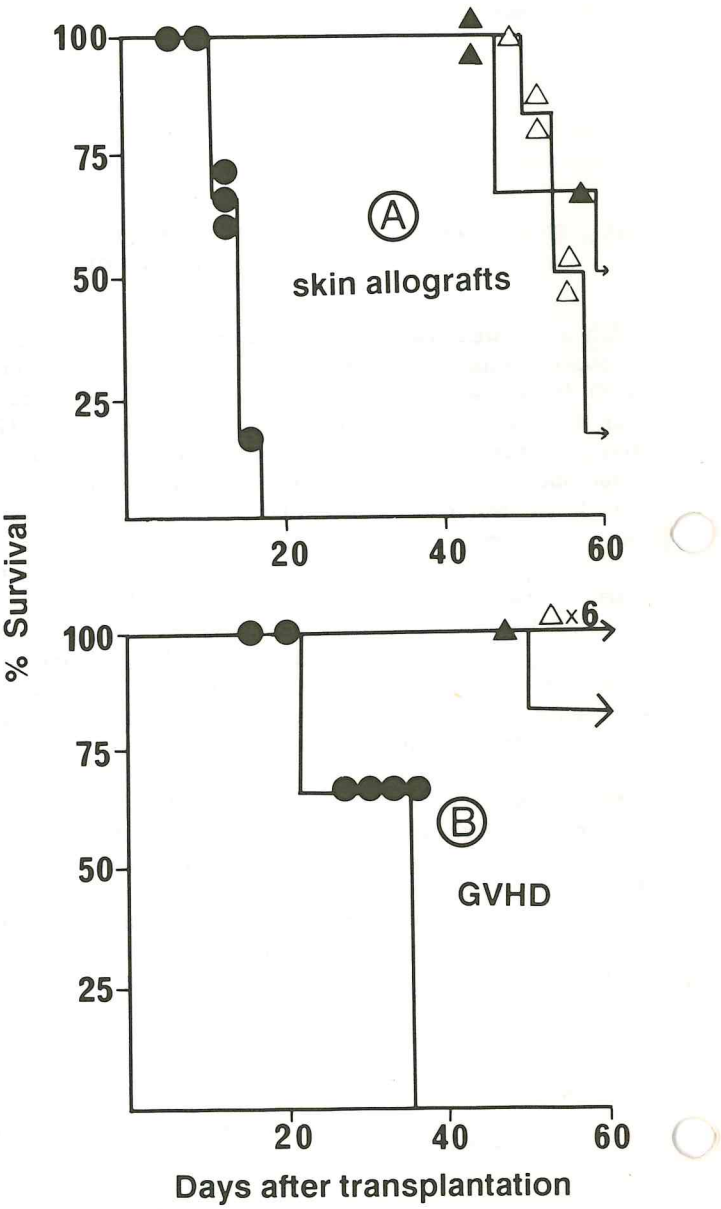

FIGURE 2. Anti–Thy-1.2 mAb suppress skin allograft rejection and GVHD due to sensitization to ATG. A) Survival of C57BL/6 skingrafts in CBA mice treated with ATG until day 40 posttransplant. CBA mice were unsensitized (△) or presensitized to ATG (●) or treated with anti–Thy-1.2 mAb (▲) so as to prevent blocking of immunosuppression during subsequent presensitization. B) GVHD mortality in (C57BL/6×CBA)F1 mice following transfer of spleen and bone marrow cells of C57BL/6 donors pretreated with ATG. Donors were unsensitized (△) or presensitized to ATG (●) or protected against presensitization by anti–Thy-1.2 mAb (▲).

Immunohistochemical studies supported these results at the cellular level. ATG-induced depletion of T cell areas in mice preinjected with sensitizing doses of ATG was reduced compared with ATG-induced depletion without sensitization. In contrast, T cell depletion was clearly more general if the mice had initially received two injections of autologous anti–Thy-1 mAb (Fig. 3). Recovery from cell depletion in spleens of control

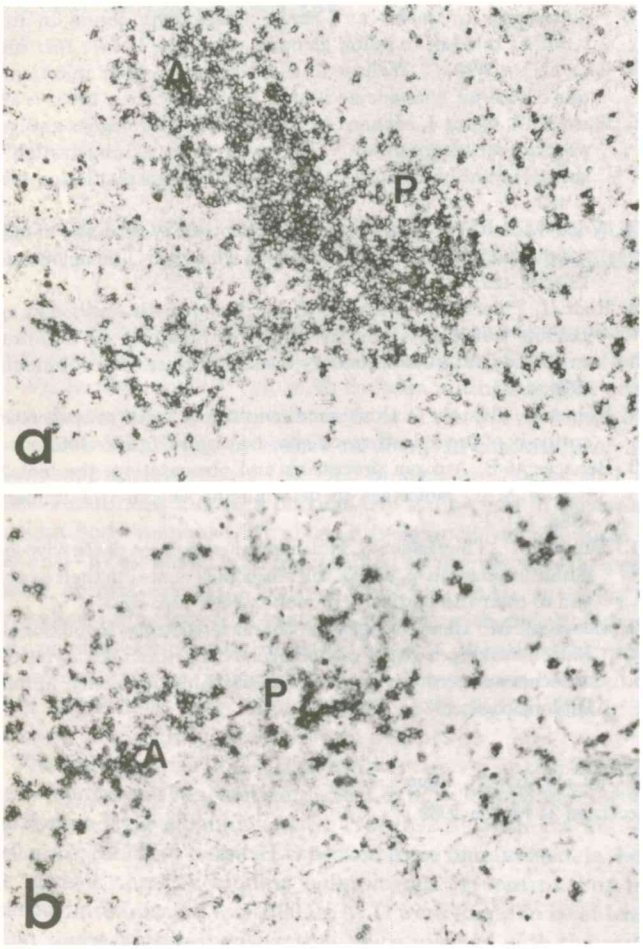

FIGURE 3. Immunohistochemical demonstration of T-lymphocyte depletion in spleen periarteriolar lymphatic sheaths (P) stained with rat anti-Ly 1 mAb (*22*) and peroxidase-labeled mouse anti-rat Ig. A = central arteriole; × 120. a) Low-grade T cell depletion by ATG in mice presensitized with ATG. b) Almost complete T cell depletion in mice injected with two doses of autologous anti–Thy-1 mAb before sensitizing ATG injections.

mice treated with the monoclonal antibody alone was found to be completed when examined 4 weeks later.

Our data thus show that antiantibodies to ALG can indeed completely block the suppression of cellular immunity in conventional murine skingraft and GVHD models. It is probably less important whether such antiantibodies have anti-idiotype (against antigen-binding–variable Ig regions) or anti-isotype (against constant Ig regions) specificity. Even if the heterogeneous idiotypes in polyclonal ALG may be regarded as less immunogenic than the homogeneous idiotype of a mAb, there are probably sufficient constant region epitopes in ALG to explain its formation of inhibitory antiantibodies. Binding of antiantibodies to the idiotypes of ALG, thereby preventing ALG from binding to T cells, must not be regarded as the only mechanism by which ALG loses its immunosuppressive potency in vivo. Binding to Ig isotypes can be equally inhibitory, also in vivo. Studies with mAb in mice reveal that antiantibodies against constant Ig region antigens accelerate the clearance of anti–T cell mAb and thereby curtail their immunosuppressive effect (*8*).

Our observation that T lymphopenia preceding ALG treatment suppresses antiantibody formation implies that antibodies in ALG that react with B lymphocytes, including the B cells with receptors for heterologous Ig, do not contribute to the immunogenicity of ALG, at least not in the absence of Thy-1–positive cells. Antiantibody formation to ALG rather follows the characteristics of immune responses to T cell–dependent antigens requiring the conjugation of antigen-processing and presenting cells with T cells (*20*). The present model of mice rendered T-lymphopenic by IgG2a anti–Thy-1.2 mAb may be informative in two further respects concerning the study of antiantibody formation: First, which is the responsible subpopulation of Thy-1–positive cells? Is it the L3T4 helper cells? Second, could the methods of induction of tolerance towards non–cell-binding heterologus antibodies (*7*) become effective also with cell-binding antibodies when applied to T-lymphopenic recipients? If anti–T cell antibodies lack their "carrier," i.e. T cells, induction of tolerance toward heterologous anti–T cell Ig, including its idiotype, might become easier and comparable to that of heterologous non–cell-binding Ig. T lymphopenia induced by agents other than heterologous antibodies preconditions the recipients to suppression of antibody formation against polyclonal but also against monoclonal heterologous antibodies. Cyclophosphamide (*8*) or sublethal irradiation (unpublished observation), for example, was found to suppress sensitization to rat anti–Thy-1 mAb in mice.

Immunosuppression induced by ALG still compares favorably with that induced by heterologous and homologous anti–T cell mAb, at least if tested under the stringent conditions of fully mismatched mouse skingraft models (*21*). It may be an argument for assembling "polyclonal" cocktails of anti–T cell mAb. Suppression of antiantibodies to polyclonal ALG may thus remain a topic of interest.

Summarizing, mice produce antibodies to ATG that can completely prevent tolerance in skin allograft and GVHD models. Antiantibody-induced blocking of immunosuppression was, however, avoided by inducing T lymphopenia with anti–Thy-1 mAb before starting the injection of otherwise sensitizing doses of ATG.

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