Adoptive Transfer of Low Zone Tolerance into Normal Adult Mice[1] (36814)

DAVID S. TERMAN, PERCY MINDEN, AND ALFRED J. CROWLE

Webb-Waring Lung Institute, National Jewish Hospital, and Departments of Medicine, Pediatrics, and Microbiology, University of Colorado School of Medicine, Denver, Colorado 80220

Exposing adult animals to small doses of an antigen may make them immunologically tolerant of it (1–3). This effect as observed in mice for such purified protein antigens as bovine serum albumin (BSA) has been termed "low dose tolerance" (4). Low dose tolerance can be elicited by repeated injections of BSA ranging from 10 μg to 10 ng (5). Similar specific, lasting unresponsiveness also has been observed following single intravenous injection of antigen in adult mice (6) and rabbits (7, 8), and after a series of subimmunogenic intravenous injections in rabbits (9).

That small doses of antigen can tolerize suggests explanations for low dose tolerance independent of classic clonal inactivation (10). For example, it might be due to replacement of immunocompetent cells by tolerant cells (9), or represent suppression of cell replication by newly synthesized antibody (7). Although not indicating how humoral antibodies may act immunosuppressively, the experiments described below do contribute to steadily mounting evidence for their importance in tolerance phenomena and specifically, here, in low dose tolerance.

Materials and Methods. CAF_1 female mice (Jackson Laboratories) were used. Techniques employed for bleeding these animals, preparing suspensions of various kinds of viable cells from them, X-irradiating them, immunizing them with BSA (crystalline, Pentex), and measuring their serum antibodies by primary interaction with ^{125}I-BSA have been described (11). However, the latter is redescribed again here because of its importance to interpreting the results presented. Thus, the antibodies were measured by the capacities of antisera from individual mice to bind radiolabeled BSA N, and expressed as ABC-33 values representing the micrograms of ^{125}I-BSA which could be bound by 1 ml of undiluted antiserum at an antigen concentration of 0.02 μg N. The serum antigen-binding capacity was calculated by determining the ultimate dilution at which it could bind 33% of the 0.02 μg N ^{125}I-BSA mixed with it. An ABC-33 endpoint was not reached if an antiserum failed at any dilution to bind 33% of the labeled antigen. This indicated low or sometimes no binding activity.

Because all sera were stored frozen so that their antibody titers could be determined at the same time although they were drawn at different times, the same values for tolerant and nontolerant control mice will be found in Figs. 2, 3, and 4. Three-times crystallized ovalbumin (OVA) was purchased from Nutritional Biochemicals Corp.

Tolerizing solution consisted of 1 mg/ml of BSA in physiologic saline diluted to 20 μg/ml in saline containing 1% normal mouse serum (5). It was divided into 20 ml volumes, stored at —20°, and individual portions thawed only once for use. Six-week-old mice were made tolerant by intraperitoneal (ip) injections of 0.5 ml volumes of the solution on Monday, Wednesday, and Friday of each week for 10 consecutive weeks. Then they were rested for 3 wk before being used.

Experiments and Results. Low dose tolerance can be transferred adoptively with spleen cells into lethally X-irradiated recipients. Spleen cells (12×10^7) from tolerant mice were transferred intravenously (iv) into each of four syngeneic recipients which 15

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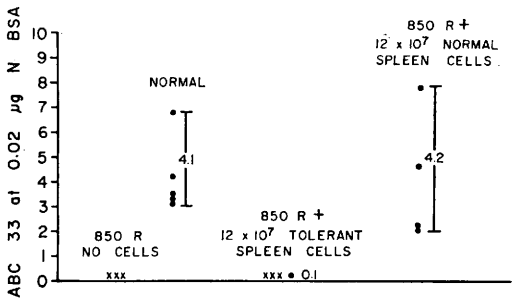

FIG. 1. Transfer of low zone tolerance into lethally irradiated mice with tolerant donor spleen cells. Mean ABC-33 values were determined for sera taken 3 wk after challenge. "X" data points in this and Figs. 2–4 indicate sera which did not have enough antibody to reach the ABC-33 end point.

min before had received 850 R. At the same time other irradiated recipients were injected with 12×10^7 normal splenocytes. These two groups, untreated control mice, and mice only irradiated, next were immunized subcutaneously (sc) with 5 mg BSA in incomplete Freund adjuvant (IFA) (11). They were sacrificed 3 wk later and their sera tested for antibodies. The irradiated recipients of tolerant spleen cells exhibited impaired immunologic responses compared with the irradiated recipients of normal spleen cells, which responded to BSA like unirradiated control mice (Fig. 1).

Although indicating tolerance in the transferred cells, this experiment did not distinguish between whether transferred cells could not react to antigen for lacking responsive clones or because they were actively suppressed. If the former, then 12×10^7 normal spleen cells should restore responsiveness to low zone tolerant mice as they can to lethally irradiated mice. Therefore, such transfer was effected iv into low dose tolerant mice. Four days later these recipients and appropriate control groups of mice were immunized sc with 25 μg BSA in IFA, and 5 wk later their sera were tested for antibodies. The results (Fig. 2) demonstrate that the transferred cells were unable to break tolerance. Similar transfer of either thymus cells or a mixture of these and bone marrow cells gave equivocal results, for recipients of both types of suspension responded better than tolerant mice but not as well as nontolerant controls.

Whereas normal spleen cells will not break tolerance, spleen cells from immunized donors sometimes can (11–15). The experiment above was extended to include iv transfer of 12×10^7 spleen cells from donors immunized with 250 μg BSA in IFA 8 wk previously into tolerant mice 3 wk after their last tolerizing injection. These recipients did evince some increase in response to immunization over untreated tolerant mice, but they did not make as much antibody as normal recipients of such cells (Fig. 3).

The suggestion of Figs. 2 and 3 that low dose tolerance is an active suppression of immunocyte response to antigen is supported also by the following data. Tolerant splenocytes (8×10^7) obtained from mice 3 wk after their 10-wk tolerizing treatment were transferred ip into normal recipients, and then these were immunized with 25 μg BSA in IFA. Their sera taken 5 wk later and compared with sera from control groups indicate that low dose tolerance had been adoptively transferred (Fig. 4). This transfer is probably antigenically specific, since normal recipients of 8×10^7 spleen cells taken from donors tolerant to BSA responded normally to injection of 100 μg OVA in IFA as shown in Table I.

Discussion. These experiments indicate that normal splenocytes which can restore immunologic responsiveness to X-irradiated mice do not break low zone tolerance in mice. Immune splenocytes, normal thymocytes, and a mixture of these thymocytes and bone marrow cells do weaken this tolerance. The tolerance, apparently antigenically specific, was transferred adoptively into both

TABLE I. Response to OVA of Normal Recipients of Tolerant Splenocytes.[a]

	Normal recipients of tolerant cells	Normal controls
	12.6	8.9
	10.5	8.2
	9.2	7.3
	3.9	3.6
Mean	9.0	7.0

[a] In ABC-33 values 5 wk after challenge with 100 μg OVA in IFA.

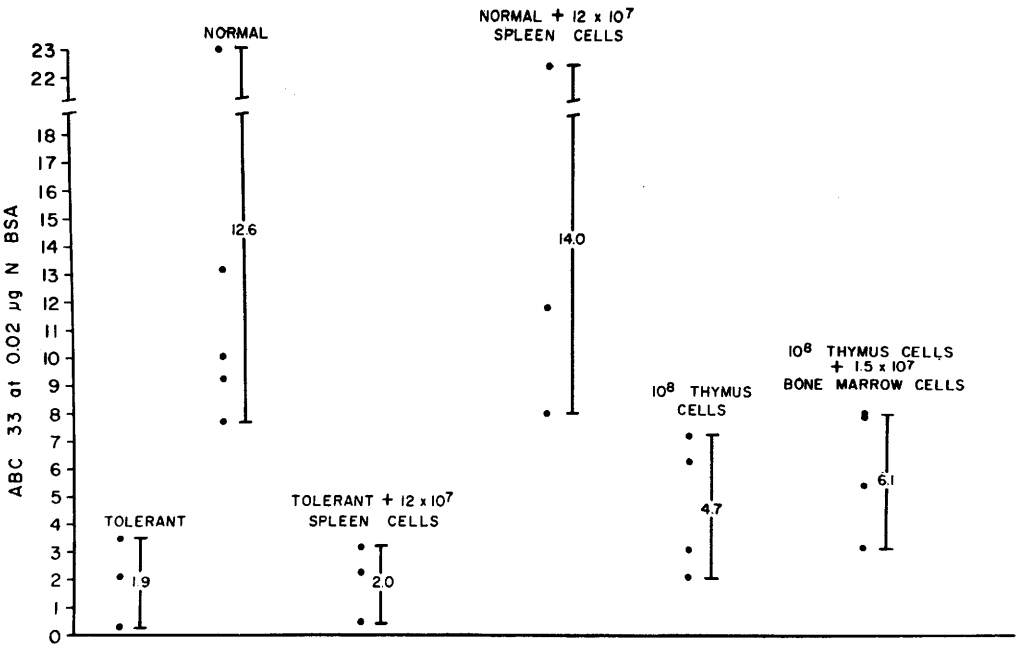

FIG. 2. Inability of normal spleen cell transfer to break low dose tolerance. Transfers of thymus cells or of these and bone marrow cells seem to have diminished it. Mean ABC-33 values for this and Figs. 3 and 4 were determined for sera taken 5 wk after challenge.

X-irradiated and fully competent normal recipients.

These findings suggest that low zone tolerance is not a clone-loss phenomenon. More likely, it is due to the presence of an active specific suppressor (*e.g.*, enhancement-like antibody) of primary immunologic activation in tolerant mice. Adoptive transfer of tolerance into normal recipients[2] probably was not due to transfer of antigen and a resulting active induction of tolerance. With its short half-life and low dose (300 μg total), insufficient BSA should have been present in tolerant mice at the time of transfer. In addition, since induction of low zone tolerance is gradual and requires cumulative exposure to antigen (5), a single injection of so minute a quantity of BSA as might have been present probably could not have tolerized normal mice.

Adoptive transfer might have been due to the transfused tolerant spleens containing numerous antigen-binding cells incapable of secreting antibody but able to combine with and divert antigens from stimulating antibody-making cells. Sjöberg (16) showed spleens from mice tolerant to *Escherichia coli* polysaccharide to contain an increased number of such antigen-binding cells. This explanation cannot be ruled out by our data, but quantitatively it seems unlikely.

The most attractive explanation for adoptive transfer of immunosuppression in these experiments is that the tolerant splenocytes were secreting a tolerance-maintaining antibody. Roles for antibodies in regulating and suppressing immune responses are now well established (17) and recently have been incorporated into explanation of several forms of tolerance hitherto thought caused by other mechanisms (18, 19). The results reported here are reminiscent of the enhancement phenomenon which can be adoptively trans-

[2] Transfer of tolerance into irradiated recipients has been effected easily by several investigators but has only modest definitive meaning. Since the irradiated recipients essentially are passive "test tubes" which do little more than support the life and activities of donor cells, such transfer does not indicate whether the tolerance is active or passive.

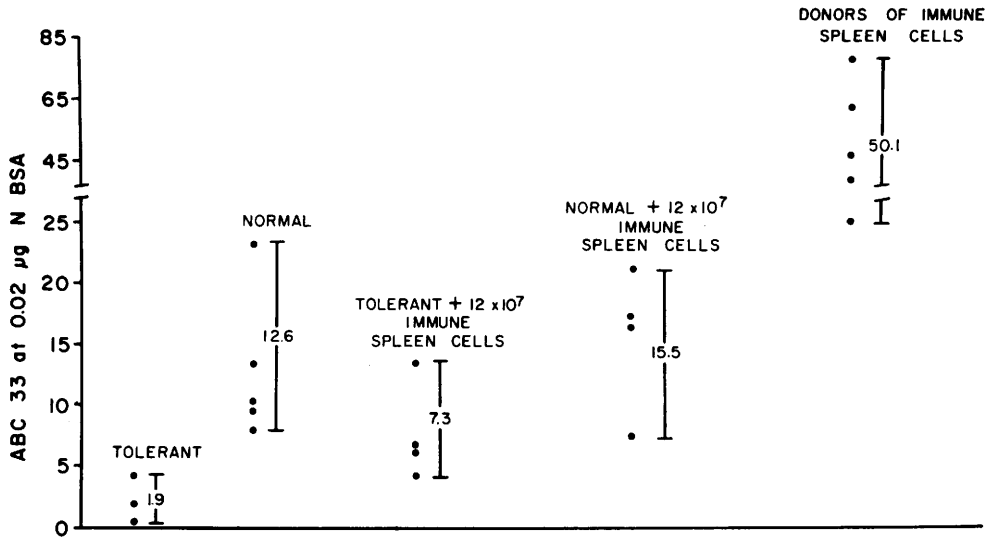

FIG. 3. Capacity of transfused immune splenocytes to lessen but not abrogate low zone tolerance.

ferred to normal syngeneic recipients to supress cell-mediated immunity (20, 21). Analogous transfers have been reported for both split tolerance (12) and Sulzberger-Chase tolerance (22) models in mice. Antibody has been implicated as an effector in neonatal homograft tolerance in mice (20, 24) and *in vitro* unresponsiveness to flagellin (25). We have shown recently that it may also be responsible for neonatal tolerance to purified protein antigen (11). An immunosuppressive effect by antibodies at

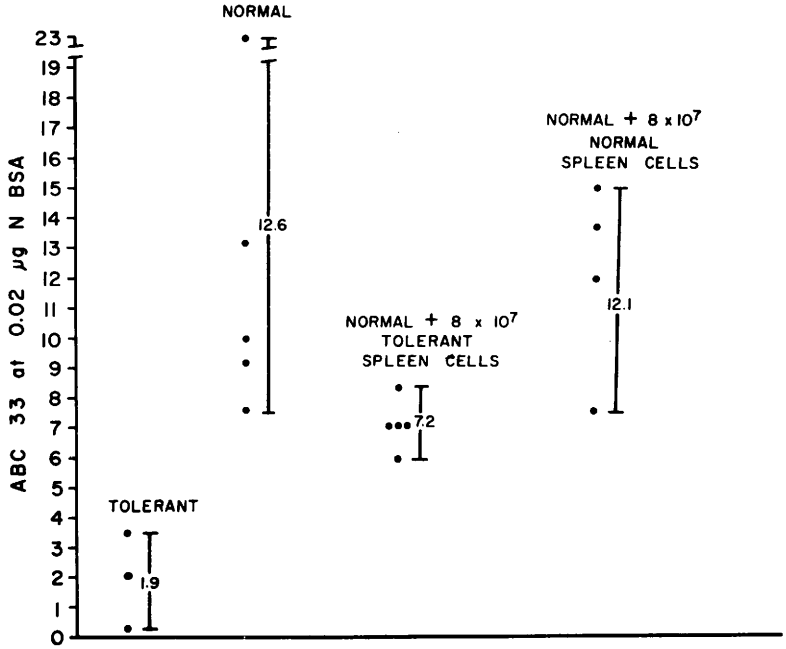

FIG. 4. Capacity of tolerant spleen cells transferred into normal mice to suppress immunologic responsiveness.

least partly accounting for low dose tolerance therefore should not be unexpected.

Summary. Low zone tolerance to BSA in CAF_1 mice appears to be more a specific active immunosuppressive effect than an immunocyte clone-loss phenomenon. Quantities of normal syngeneic splenocytes able to immunologically reconstitute lethally X-irradiated mice failed to break the low zone tolerance, and similar cells obtained from immunized donors did so only partially. Furthermore, spleen cells were able to transfer this tolerance adoptively not only into immunologically deficient X-irradiated recipients but also into immunologically intact normal recipients. These data suggest that this low zone tolerance may be an example of antibody-mediated immunosuppression.

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