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A role for suppressor T cells in induction of self-tolerance

(ontogeny/autoimmunity)

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ABSTRACT **The potential role of suppressor T cells (T_s) in the induction of self-tolerance was investigated by eliminating I-J$^+$ cells during ontogeny (I-J antigens are encoded by the *I-J* subregion of the murine major histocompatibility complex). To achieve this, F_1 mice were exposed to anti-I-J antibodies via the transplacental route by mating B10.A(3R) females, preimmunized with B10.A(5R) cells, with CBA males. At 6 weeks of age, the offspring were injected with rat erythrocytes (RRBC) to induce erythrocyte autoantibodies. By comparison with age-matched controls, T_s-depleted mice produced significantly higher titers of autoantibody, whereas there was no difference in the antibody response of the two groups to the foreign determinants on the RRBC. The selective increase in autoantibody production was mirrored at the clonal level by the appearance of self-reactive B-cell hybridomas after fusion of RRBC-immune spleen cells with the NS-1 cell line. On the other hand, when helper cell function of RRBC-primed cells was measured in a T-cell proliferative assay, T_s depletion *in utero* resulted in enhanced T-cell activity to nonself (RRBC) but not to self (mouse erythrocyte) determinants. Thus, helper T cells recognizing nonself determinants on RRBC appeared to be responsible for activating self-specific B cells, presumably through linked recognition of different epitopes on mouse erythrocytes. Taken together, these findings indicate that elimination of I-J$^+$ cells during ontogeny can lead to the appearance and activation of "forbidden" B-cell clones and points to a central role for T_s in induction as well as maintenance of self-tolerance.**

A precise understanding of autoimmunity depends on identification of the mechanisms responsible for the induction and maintenance of self-tolerance. Despite substantial advances made in delineating the events underlying the development of tolerance to foreign antigens, a consensus has yet to be reached concerning the cellular basis of self-tolerance. Protagonists of the clonal deletion theory (1) and its more recent modifications (2) base their arguments for a repertoire-purging mechanism on the exquisite sensitivity of immature lymphocytes (e.g., pre-B-cells) to physiological concentrations of antigen (2). On the other hand, there are a number of experimental observations that are not compatible with a simple deletion model of self-tolerance but rather implicate the existence of active suppressor mechanisms capable of inhibiting autoimmune responses. For example, autoantigen binding B cells (3) and autoreactive T helper (T_h) cells (4) have been demonstrated in healthy subjects, and it is relatively easy to induce autoimmune disease in normal animals with self antigens in adjuvant (5). Furthermore, suppressor T cells (T_s) with specificity for a variety of self antigens (6–10) have been found. At present, however, it remains unclear whether antigen-specific T_s serve only as a fail-safe mechanism for maintenance of self-tolerance or whether they are involved in inductive events during ontogeny as well. In an attempt to study this question, a model was established for eliminating T_s *in utero*. This involved exposure of fetal mice via the transplacental route to maternal IgG antibodies with specificity for the serologically defined "I-J" determinant encoded by the *I-J* subregion of the murine major histocompatibility complex (MHC) (11). On subsequent testing in adult life, anti-I-J-exposed progeny were more prone to development of autoantibody responses against autologous erythrocytes than were untreated controls.

The precise structure and genetic origin of the I-J determinant is still controversial (12, 13). In particular the *I-J* gene(s) do not appear to be typical of other class II histocompatibility genes. Nevertheless, despite present uncertainties concerning the physiological role of I-J (14), there is sound experimental evidence demonstrating that anti-I-J antibodies can be used to abrogate T_s function selectively without influencing the activities of other T-cell subsets (15, 16). Thus, the demonstration here of increased susceptibility of anti-I-J-exposed mice to the induction of autoimmunity is consistent with a central role for T_s in mediating self-tolerance.

MATERIALS AND METHODS

Animals. Inbred mice of the CBA/Ca/T6, B10.A(3R), B10.A(5R), and BALB/c/J strains were used. Their genetic purity was confirmed by isoenzyme analysis (P. Baverstock, South Australian Museum, Adelaide, South Australia).

Experimental Model for Eliminating T_s During Embryonic Life. T_s can be distinguished from other T-cell subsets by the fact that they carry the serologically defined I-J determinant (11) and, thus, can be selectively removed by antibodies with anti-I-J specificity (16). B10.A(3R) (*I-J^b*) females were hyperimmunized with six injections of 10^7 spleen, lymph node, and thymus cells from B10.A(5R)(*I-J^k*) female donors at weekly intervals in order to generate circulating IgG anti-*I-J^k* antibodies. Immediately after the final injection, the B10.A(3R) females were mated with CBA(*I-J^k*) males, thus exposing the F_1 progeny to anti-I-J antibodies *in utero* and during lactation. The efficacy of this regimen was confirmed by showing that maternal serum from immunized but not control animals could eliminate human gamma globulin (HGG)-specific T_s in our standard assay for suppression (15). Each female was mated only once to avoid possible spontaneous production of anti-I-J antibodies by repeated exposure to MHC antigens on the placental trophoblast. At 6 weeks of age, F_1 offspring born to anti-I-J antibody-positive mothers and control animals (i.e., normal F_1 mice) were tested for their ability to mount autoantibody and proliferative responses to autologous erythrocytes.

Antibody Production to Autologous and Rat Erythrocytes (RRBC). [B10.A(3R) × CBA] F_1 mice born to anti-I-J^k antibody-positive mothers and normal F_1 controls were

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Abbreviations: DAGT, direct anti-globulin test; HGG, human gamma globulin; MHC, major histocompatibility complex; MRBC, mouse erythrocytes; RRBC, rat erythrocytes; SRBC, sheep erythrocytes; T_h, helper T cells; T_s, suppressor T cells.

immunized with a single intraperitoneal injection of 2×10^8 normal RRBC which carry not only rat-specific antigens but also determinants with cross-reactivity for mouse erythrocytes (MRBC) as well (17). Blood was collected at intervals thereafter for measurement of antibodies to MRBC and RRBC.

Autoantibodies bound to MRBC were measured by the direct anti-globulin test (DAGT). Briefly, doubling dilutions of rabbit anti-mouse Ig were added to 25 μl of a 2% solution of washed MRBC in round-bottom microtiter trays. The DAGT was expressed as the mean $\log_2$ titer (±SEM) of the highest dilution of rabbit anti-mouse Ig to cause macroscopic hemagglutination. Serum anti-RRBC antibodies were measured by hemagglutination using a 2% solution of washed RRBC and doubling dilutions of mouse sera. Results were again expressed as mean $\log_2$ titer (±SEM) of the highest serum dilution to cause macroscopic hemagglutination.

Preparation and Usage of Anti-I-J^k Antiserum. Anti-I-J^k antiserum was prepared by the protocol used for the generation of these antibodies in prospective B10.A(3R) mothers (see above). In the current experiments, 0.7 ml was injected intravenously into normal F_1 recipients at 6 weeks of age. Its potency was tested by showing that *in vivo* administration of between 0.5 and 1.0 ml of this antiserum abrogated memory T_s in the well-characterized HGG system (15).

Production and Screening of B-Cell Hybridomas. Adult F_1 progeny of anti-I-J antibody-positive and normal control mothers were immunized three times with 2×10^8 RRBC at weekly intervals. Four days after the final immunization, immunized spleen cells were fused with NS-1 cells as described elsewhere (18). Supernatants from the resulting hybrids were screened for antibodies to both MRBC and RRBC by using a cellular RIA. For this purpose 50 μl of hybridoma supernatant was added in duplicate to 10^7 washed MRBC or RRBC (in 50 μl of phosphate-buffered saline) in flexible polyvinyl chloride V-bottomed microtiter plates (Dynatech, Alexandria, VA), which previously had been blocked with 1% bovine serum albumin in phosphate-buffered saline. After incubation for 75 min at 37°C, plates were washed three times, and 25 μl of ^{125}I-labeled sheep anti-mouse Ig (≈75,000 cpm) was added to each well. After a further 75-min incubation, plates were washed again, then dried, and counted. Positive supernatants were defined as those with a specific activity >2 times that of control supernatants containing monoclonal antibodies of irrelevant specificity.

***In Vitro* Proliferative Responses to MRBC and RRBC.** Anti-I-J antibody-exposed and control F_1 mice were immunized with 10^6 RRBC emulsified in an equal volume of complete Freunds adjuvant (Commonwealth Serum Laboratories, Melbourne, Australia) in both hind-foot pads and at the base of the tail. Fifteen days later draining lymph nodes from a minimum of four animals were removed under sterile conditions, pooled, and sieved to yield a single-cell suspension. Triplicate cultures were then established in round-bottom microtiter plates (Nunc) containing 10^6 lymph node cells and various numbers of either MRBC, RRBC, or sheep erythrocytes (SRBC) in RPMI 1640 medium (Flow Laboratories, Sydney, Australia) supplemented with 10% human group A serum, sodium bicarbonate (0.85 g/liter), 25 mM Hepes, 2 mM glutamine, 50 μM 2-mercaptoethanol, penicillin, and streptomycin in a final volume of 200 μl. After incubation in 5% CO_2/95% air for 5 days and 18 hr before harvesting, 0.5 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine (Amersham, Bucks, U.K.) was added to each well. Incorporated radioactivity was expressed as the arithmetic mean of disintegrations per minute, and stimulation indices were compared to those of cultures without added erythrocytes.

Statistical Analysis. Student's *t* test was used for statistical comparison between groups.

RESULTS

Effect of T_s Depletion *in Utero* on Anti-Erythrocyte Autoantibody Production. [B10.A(3R) × CBA] F_1 mice born to anti-I-J^k antibody-positive mothers and control offspring were found to be DAGT-negative at 6 weeks of age. They were immunized once with 2×10^8 RRBC, and the DAGT titers were measured 2, 4, and 6 weeks later. This immunization protocol was chosen to optimize the sensitivity of the system and was based upon the results of preliminary experiments in which it was shown that at least two to three injections of RRBC were required to induce a significant autoantibody response in normal mice. In the current experiments (Fig. 1) minimal autoantibody production was observed in the control F_1 offspring, only one of six animals being DAGT-positive on a single occasion. By contrast, all anti-I-J antibody-exposed mice developed significant autoantibody levels, which remained elevated for the duration of the study. Titers in test and control animals were significantly different at all time points analyzed ($P < 0.001$).

Differential Effect of T_s Depletion *in Utero* on Production of Anti-Erythrocyte Autoantibodies Versus Anti-RRBC Antibodies. To investigate whether the effect of anti-I-J antibody exposure during ontogeny was restricted to autoantibody production, a second series of experiments was designed in the same way except that the antibody responses to RRBC (nonself) as well as MRBC (self) were measured 2 and 5 weeks after a single injection of RRBC. Once again there was a significant difference in mean DAGT titers between F_1 mice exposed to anti-I-J antibodies *in utero* and normal controls at both time points tested (Fig. 2, $P < 0.05$ and $P < 0.01$, respectively). Thus, all animals ($n = 12$) produced anti-erythrocyte autoantibodies, whereas only 7 of 17 and 8 of 15 normal F_1 animals were DAGT-positive 2 and 5 weeks after challenge. By contrast comparable anti-RRBC antibody responses were observed in both test and control groups that were not statistically different at the time points analyzed. Similar results were obtained in a total of five experiments designed in the same way. Therefore, these findings are consistent with a selective effect of maternal anti-I-J antibodies on anti-erythrocyte autoantibody production in the F_1 offspring.

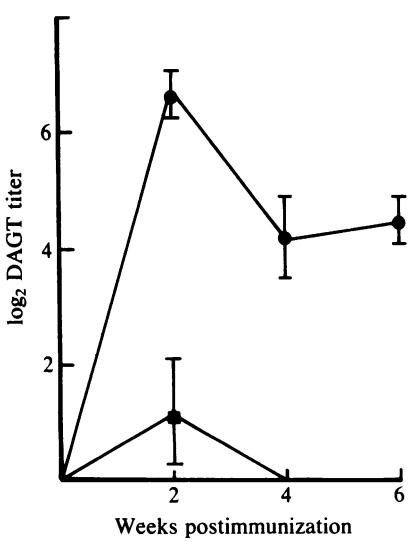

FIG. 1. Comparison of anti-erythrocyte autoantibody responses in [B10.A(3R) × CBA] F_1 mice exposed to anti-I-J antibodies during ontogeny (●) and normal F_1 controls (■). Autoantibody levels were measured by DAGT and expressed as the mean (±SEM) of the $\log_2$ titer of the highest dilution of rabbit anti-mouse Ig to cause macroscopic hemagglutination. A significant difference in DAGT titers was observed between test and control groups at all time points analyzed ($P < 0.001$).

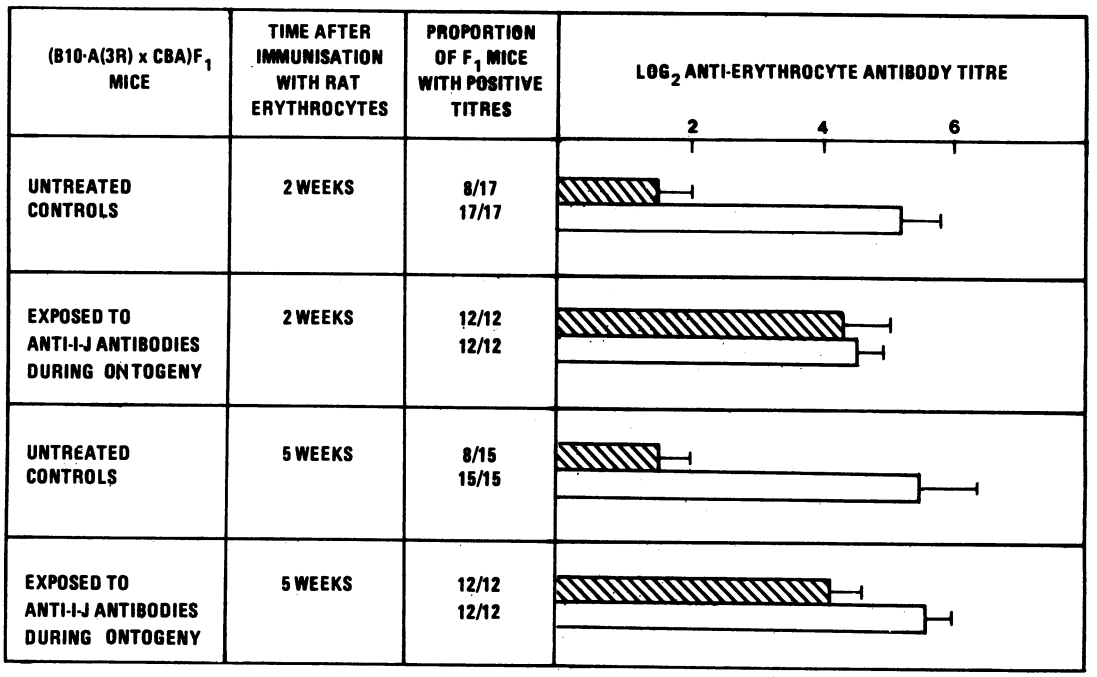

FIG. 2. Comparison of anti-MRBC and anti-RRBC antibody responses in [B10.A(3R) × CBA] F_1 mice exposed to maternal anti-I-J antibodies during ontogeny and normal F_1 controls. Hatched bars represent DAGT titers, and open bars represent serum anti-RRBC antibody titers. A significant difference in DAGT titers was observed between test and control groups at both time points tested ($P < 0.05$ and $P < 0.01$, respectively), whereas the anti-RRBC titers were similar.

It could be argued that the enhanced autoantibody production observed here resulted from depletion of self-reactive T_s, not during embryonic life but postnatally because of the presence of residual maternal anti-I-J antibodies in the circulation of the F_1 offspring. Since the amount of anti-I-J antibodies would have fallen to low levels by 6 weeks of age, this possibility was tested by injecting an excess of anti-I-J^k antiserum (0.7 ml) into normal F_1 mice. Twenty-four hours later these animals and a group of recipients of normal B10.A(3R) serum were challenged once with the standard dose (2×10^8) of RRBC. There was no statistical difference in either autoantibody or anti-RRBC titer between test and control groups (data not shown), thereby excluding a major role for residual maternal anti-I-J antibodies in the postnatal period.

Effect of T_s Depletion *in Utero* on the Frequency of Hybridomas Secreting Anti-Erythrocyte Autoantibodies. The demonstration of a selective increase in anti-erythrocyte autoantibody production following T_s depletion *in utero* raised the possibility that self-reactive B cells as well as B cells making antibodies to cross-reactive determinants had emerged during ontogeny. To test this directly, B-cell hybridoma cultures derived from fusions between NS-1 cells and RRBC-primed spleen cells from normal or anti-I-J antibody-exposed donors were screened for antibody activity to MRBC and RRBC prior to cloning. In the case of normal donors, hybridomas were found to produce only RRBC-specific or cross-reactive antibodies but no anti-MRBC antibodies (Fig. 3). The designation of cross-reactivity was confirmed after limit dilution analysis, when all clones from cross-reactive cultures remained cross-reactive, thus excluding the possible presence of hidden anti-self clones (data not shown). By contrast anti-I-J-exposed donors yielded a significant number (15%) of hybridomas with specificity for self erythrocytes, whereas the proportion of cross-reactive clones (24%) was reduced compared to that obtained in normal cultures (95%). In addition, a marked increase in the number of RRBC-specific hybridomas occurred. The average level of binding to MRBC-specific determinants was low (2.5–5 times background) compared with that to RRBC or cross-reactive determinants (5–26 times background) as measured by cpm bound in the cellular RIA (data not shown).

Effect of T_s Depletion During Ontogeny on the *in Vitro* Proliferative Response to MRBC and RRBC. The results to date are consistent with a role for T_s in preventing the emergence of autoreactive B cells during ontogeny but do not tell us whether T_s can influence developing T_h cells in a similar way or how exposure to RRBC results in preferential activation of B cells with unique self specificity. To examine

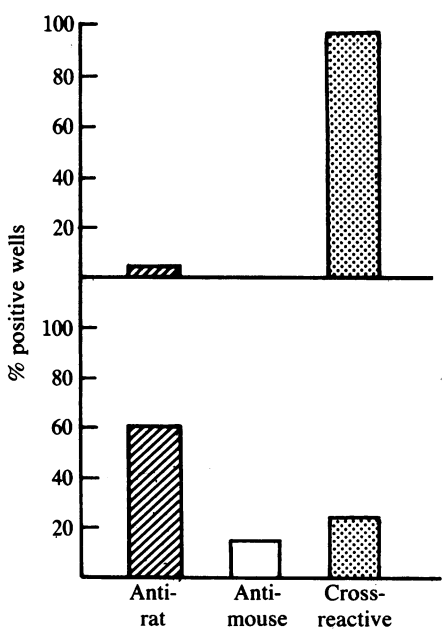

FIG. 3. Comparison of the frequencies of B-cell hybridomas secreting antibodies to MRBC, RRBC, or cross-reacting determinants found after fusion of NS-1 cells with RRBC-primed spleen cells from anti-I-J antibody-exposed F_1 mice (*Lower*) and normal BALB/c/J controls (*Upper*). Positive supernatants were defined as those giving >2.5 times the background cpm in the cellular RIA.

these issues, the *in vitro* proliferative response to MRBC and RRBC was measured. After priming *in vivo* with RRBC, lymph node cells from anti-I-J antibody-exposed and control mice were stimulated in culture with MRBC, with RRBC, or with SRBC that lack cross-reactive determinants. At the highest antigen dose (10^7 erythrocytes per well), a significant increase in proliferation to RRBC was obtained with cells from T_s-depleted donors compared with controls, whereas the response to MRBC measured in terms of absolute dpm above background was similar in the two groups despite a 2-fold difference in stimulation indices (Table 1). On the other hand, no significant change in reactivity to the irrelevant antigen, SRBC, was observed, which suggests that the T-cell response has some degree of specificity and is directed predominantly towards the nonself determinants on RRBC.

DISCUSSION

The existence of autoreactive T_h and B cells (3, 4) in normal animals provides an *a priori* argument in favor of a role for active suppressor mechanisms in regulating antiself responses. Experimental evidence supporting the involvement of T_s in self-tolerance comes from a number of different approaches. First, T_s have been found in several models of neonatal tolerance (19–21). Second, recent studies have led to the demonstration of T_s with specificity for a wide range of cell-associated and soluble self antigens (6–10, 22, 23). Third, the introduction of a foreign antigen such as human IgG (HGG) into the embryo via the transplacental route so that it mimics self during ontogeny has been shown to result in postnatal development of both tolerance and specific-effector and memory T_s bearing the phenotype Ly-2^+, Ia $(I\text{-}J)^+$ (24). Memory T_s differ from memory T_h in that they remain dormant until activated by antigen in immunogenic or tolerogenic form and, therefore, may be overlooked in classical mixing experiments. In the HGG model, the memory cell population was readily detected up to 16 weeks after birth and exerted a profound inhibitory effect on reexposure of the offspring to the same antigen in adult life. Furthermore, antenatal exposure to anti-Ly-2 and anti-I-J antibodies has been shown to prevent the induction of tolerance as well as suppression (unpublished observations). On this basis it would be predicted that true self antigens would induce memory T_s preferentially *in utero* so that a pool of long-lived T_s would be present from birth and would be renewed throughout life by constant exposure to self antigens.

The above findings are consistent with a role for memory T_s as a fail-safe mechanism in maintenance of self-tolerance but do not conclusively establish whether T_s are *ipso facto* the principal mediators of self-tolerance. In an attempt to resolve this issue, fetal mice were exposed to maternal anti-I-J antibodies of the appropriate specificity ($H\text{-}2^k$) via the transplacental route. Subsequently the T_s-depleted progeny were tested for a loss of self-tolerance in functional assays of autoimmunity. Direct confirmation of a quantitative reduction in T_s by fluorescence-activated cell sorter analysis of $I\text{-}J^k$-positive cells at birth was impracticable since the intensity of fluorescence with antisera and a monoclonal antibody of anti-$I\text{-}J^k$ specificity was insufficient to discriminate between cells from anti-I-J-exposed and control mice. Nevertheless, as shown by the results of the functional assays, mice exposed to anti-I-J antibodies *in utero* were clearly more susceptible to autoimmunization than were untreated controls.

Experimental autoimmune hemolytic anemia can be induced by repeated injections in normal mice of RRBC that carry cross-reacting determinants on their membrane and is a good choice of model for studying suppression in self-tolerance on two counts. First, the production of pathogenic autoantibodies following immunization with RRBC mimics the natural situation, where the immune system is continuously exposed to ubiquitous antigens bearing cross-reactive epitopes shared with self (2). Second, the appearance of autoantibodies is accompanied by a transient wave of T_s (7, 17). Although these cells are clearly capable of regulating the autoantibody response in adoptive transfer, it has not been established whether they play a primary role in self-tolerance or simply represent a regulatory epiphenomenon resulting from immunization with cross-reacting foreign erythrocytes. In the experiments reported here (Fig. 1), a single injection of RRBC produced a brief and barely detectable autoantibody response in control mice, whereas in age-matched animals depleted of T_s *in utero* by exposure to maternal anti-I-J antibodies, this procedure provoked a highly significant autoantibody response that was sustained for several weeks. Furthermore, the effect was specific for the antiself response, since antibody titers to foreign (rat) determinants were comparable in both test and control groups (Fig. 2).

These observations indicate that autoantibody production is selectively controlled by $I\text{-}J^+$ cells, presumably memory T_s, which are induced during early development and appear to act at the level of self-reactive B cells rather than B cells with specificity for cross-reacting determinants. The failure of an excess of anti-I-J antibody administered in adult life to influence the autoantibody response provides further evidence in favor of the involvement of self-specific memory T_s during the early stages of self-tolerance induction and excludes a significant role for residual maternal anti-I-J antibodies in the postnatal period. The lack of effect of anti-I-J exposure during ontogeny on antibody production to nonself (RRBC) may at first sight seem puzzling. The discrepancy in responses to self versus nonself can in fact be readily explained on the basis of a difference in size of the B-memory-cell pools to the two antigens. Thus, by 6 weeks of age when the experiments were performed, the reduction in

Table 1. Comparison of the *in vitro* proliferative response of RRBC-primed lymph node (LN) cells to MRBC, RRBC, and SRBC

Source of RRBC-primed LN cells	No. of erythrocytes per well	Proliferative response to erythrocytes,* dpm × 10^{-3} ± SEM		
		RRBC	MRBC	SRBC
Control mice	Nil	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
	10^5	0.9 ± 0.1 (1.1)*	1,3 ± 0.3 (1.6)	1.0 ± 0.3 (1.3)
	10^6	1.1 ± 0.1 (1.4)	1.0 ± 0.1 (1.2)	0.9 ± 0.1 (1.1)
	10^7	16.3 ± 2.7 (19.5)	9.5 ± 0.8 (11.2)	0.5 ± 0.02 (0.6)
T_s-depleted mice	Nil	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.06
	10^5	1.2 ± 0.1 (2.4)	0.5 ± 0.05 (1.0)	0.4 ± 0.05 (0.9)
	10^6	2.5 ± 0.2 (5.0)	0.6 ± 0.1 (1.3)	1.0 ± 0.2 (2.0)
	10^7	47.2 ± 1.6 (94.4)	10.8 ± 0.7 (21.7)	1.2 ± 0.2 (2.4)

*The numbers in parentheses are the stimulation indices.

self-specific suppression would have permitted the emergence of self-reactive B memory cells, whereas B memory cells with specificity for nonself would not have been generated until after exposure to RRBC at the time of challenge. In other words, the threshold of triggering of the anti-self response would have been lower than that for antibody production to foreign determinants being seen on RRBC for the first time. As a consequence, one might have expected T_s-depleted mice to display spontaneous autoantibody production in the absence of deliberate challenge with RRBC. The fact that this did not occur is presumably due to incomplete depletion *in utero* and/or partial restoration of the T_s memory cell pool in the postnatal period, when residual maternal anti-I-J antibodies were no longer detectable.

The selective increase in production of autoantibodies to MRBC following T_s depletion *in utero* was analyzed at the clonal level by comparing the specificity pattern of anti-erythrocyte antibody-secreting B-cell hybridomas after fusion of NS-1 cells with RRBC-primed spleen cells from anti-I-J-exposed or normal donors that had been deliberately primed to cross-reactive determinants on RRBC. This technique was chosen because the range of antibody specificities of the hybridoma population has been shown to reflect the specificities present in the intact animal (25). As expected, only hybridomas producing antibodies against foreign or cross-reactive determinants were obtained from normal controls primed to RRBC (Fig. 3). By contrast, a marked shift in specificity pattern occurred in hybridomas derived from anti-I-J-exposed progeny, consisting of an increase in rat-specific clones and the appearance of a significant number (15%) of B-cell hybrids with purely antiself specificity (Fig. 3). The latter finding supports the notion that T_s depletion *in utero* allows the emergence of B-cell clones with specificity for unique self determinants on autologous erythrocytes as distinct from the epitopes shared with RRBC. The lower level of binding of autoantibodies to MRBC compared with that of antibodies directed to cross-reacting or rat-specific determinants is presumably due either to low antibody avidity or to a low density of these particular self-specific determinants on the MRBC membrane.

The question then arose as to how immunization with RRBC could preferentially activate B cells with such unique self-specificity. The most plausible explanation is that T_s depletion *in utero* leads to induction of increased numbers of T_h recognizing cross-reacting or foreign determinants and that these T_h are responsible for activating self-specific B cells through linked recognition of different epitopes on autologous erythrocytes. When the specificity of T_h was examined in a proliferative assay with RRBC-primed cells from anti-I-J-exposed and control mice, T_s depletion resulted in much greater enhancement of T-cell activity to RRBC (nonself) but not to MRBC (self) (Table 1). Thus, there was no apparent evidence for emergence of self-reactive T_h nor for a significant increase in T_h-recognizing cross-reactive determinants. On this basis, T_h with specificity for foreign carrier determinants would appear to be responsible for presenting the cross-reactive haptenic determinants on RRBC to self-specific B cells. The validity of this interpretation is supported by two findings from the cell-fusion experiments (Fig. 3). First, the shift in reactivity of T_h towards nonself was paralleled by an increase in frequency of anti-rat-specific hybridomas. Secondly, the enhanced level of autoantibody production *in vivo* was due apparently to emergence of self-reactive B cells rather than to a rise in B cells synthesizing antibodies to cross-reacting determinants (Fig. 3). Nevertheless, a role for cross-reactive (or even self-specific) T_h cannot be excluded in the absence of an analysis at the clonal level because a shift in frequency of T cells recognizing either of these determinants on autologous erythrocytes could have been missed.

Taken together, the findings presented here suggest that removal of T_s during early development leads to emergence of self-reactive B memory cells that, on exposure to cross-reacting epitopes on a foreign antigen, will produce pathogenic autoantibodies. The importance of T_s in self-tolerance is further strengthened by our observations in two other models of autoimmunity. First, anti-DNA antibody production following *in vivo* stimulation with lipopolysaccharide was significantly increased after T_s depletion *in utero*. Secondly, evidence for enhanced T-cell autoreactivity to self MHC antigens was obtained in the autologous mixed lymphocyte reaction (unpublished observations). Thus, the findings in three models of autoimmunity encompassing self-reactive T cells as well as B cells imply that T_s play an important role in induction of self-tolerance during ontogeny.

Note Added in Proof. The control mice in the B-cell hybridoma experiments illustrated in Fig. 3 were of BALB/c/J origin. When normal [CBA × B10.A(3R)] F_1 mice were used instead, the frequency of clones secreting cross-reactive antibodies was decreased, and once again no anti-self-specific hybridomas were obtained from three separate fusions!

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