INDUCTION OF TOLERANCE IN VITRO BY AUTOLOGOUS MURINE TESTICULAR CELLS

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The mechanism by which self tolerance is achieved is little understood. Until recently, it was assumed that in normal individuals lymphocyte reactions against components of self did not occur. Tolerance of this kind was assumed to be based partly on the irreversible loss of lymphocytes directed against accessible self antigens, and partly on the sequestering of some self antigens from the immune system (1). Increasing evidence indicates, however, that self tolerance is a far more complex phenomenon. Lymphocytes recognizing autoantigens without leading to autoimmune reactions have been demonstrated in many experiments; for example, in normal humans, B cells bind homologous thyroglobulin (2, 3) or DNA (4), whereas in the mouse, B lymphocytes recognize distinct erythrocyte autoantigens (5, 6). Furthermore, it has been possible to sensitize T cells in vitro against syngeneic fibroblasts (7) or autologous thymus epithelium cells (8). Recently, two general mechanisms for self tolerance have been proposed: (*a*) an unresponsive state that is characterized by an irreversible loss of competent T and B lymphocytes that is maintained by the concentration of self components in the body fluid, and (*b*) a peripheral inhibition of competent lymphocytes by suppressor T cells and the products of such cells (9).

We have attempted to approach the question of the regulation of self tolerance by examining the immune response against autologous testicular cells in a mixed cell response in vitro. We studied lymphocyte reactivity against germ cells which are sequestered from the immune system versus somatic cells which are not sequestered.

We report here that under in vitro conditions, autologous germ cells are efficient inducers of tolerance by evoking suppressor T cells, whereas autologous somatic cells of the testis are immunogenic.

Materials and Methods

Animals. Young adult male mice (8–12 wk of age) were used throughout all studies. The inbred strains A/J, C57BL/6 (B6),[1] BALB/c, CBA, and AKR were purchased from The Jackson Laboratory (Bar Harbor, Maine) or were supplied from the breeding facilities of the Sloan-Kettering Institute for Cancer Research, New York.

The sterile mutants W/W^v were bred in our own mouse colony. They are F_1 mice deriving

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[1] *Abbreviations used in this paper:* B6, C57BL/6; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; SI, stimulation index; TeI, a suspension of free extratubular cells (fibroblasts, lymphoid cells, and connective tissue elements) and 15–20% of the androgen-secreting Leydig cells; TeII, a suspension of germ cells at all stages of spermatogenesis and Sertoli cells.

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from the cross C57BL/6J-W^v/+ × WB/REJ-W/+. Wild-type (+/+) animals of this cross are normal and were used as controls.

Cell Preparations. Animals providing the responder cells were ether anesthetized and bled. The blood was collected and used as a source for autologous serum, which was heat inactivated for 30 min at 56°C. The other animals were killed by cervical dislocation. Spleen cell suspensions were prepared in cold phosphate-buffered saline (PBS) in a loosely fitting glass homogenizer. The erythrocytes were lysed by brief hypotonic shock treatment. Testicular cell suspensions were prepared in two different ways: either by protease treatment or mechanical treatment. Sequential enzymatic dissociation was performed according to the modified procedure of Romrell et al. (10). The testes were decapsulated, slightly teased apart, and incubated at 37°C for 12 min in 0.1% collagenase (Worthington Biochemical Corp., Freehold, N. J.) dissolved in PBS. Thorough pipetting denuded the seminiferous tubules and yielded a suspension of free extratubular cells (fibroblasts, lymphoid cells, and connective tissue elements) and ~15–20% of the androgen-secreting Leydig cells (designated as TeI). The tubular segments were then broken up by a 15-min incubation at 37°C in 0.025% trypsin (Grand Island Biological Co., Grand Island, N. Y.) that was dissolved in Ca^{++}-ion- and Mg^{++}-ion-free PBS, to release germ cells at all stages of spermatogenesis and Sertoli cells (designated as TeII). The different cell types were identified by light microscopy according to their morphological characteristics (10). 3–6 × 10^6 TeI cells and 18–25 × 10^6 TeII cells per mouse were obtained by protease treatment. For mechanical dissociation of testicular cells, the decapsulated testes were gently teased apart and pipetted several times in PBS without Ca^{++} and Mg^{++} plus 0.2 mM EDTA. This treatment released mostly interstitial cells. Sertoli cells and germ cells were obtained by homogenizing the seminiferous tubules. Mechanical cell dissociation yielded 3–5 × 10^6 TeI cells and only 5–10 × 10^6 TeII cells per pair of testes. Because of the better yield of cells and the more gentle treatment, for most of the experiments, testicular cells were obtained by protease treatment, unless otherwise stated. For cultivation, the single cell suspensions were washed once and resuspended in RPMI-1640 medium (Grand Island Biological Co.), supplemented with 1% autologous mouse serum and 0.05 mM 2-mercaptoethanol.

Elimination of Lymphocyte Subclasses. Suppressor T cells were eliminated by incubation of 40 × 10^6 spleen cells/ml with monoclonal anti-Ly-2.2 (1:200 final dilution) for 30 min at 4°C followed by a 30-min incubation with selected rabbit complement at 37°C as described by Shen et al. (11). This procedure was repeated once. Cell recovery after anti-Ly-2.2 treatment was two-thirds of the initial cell number. Elimination of Thy-1.2-positive T cells was performed twice with monoclonal antibody against Thy-1.2 antigen. 35 × 10^6 spleen cells/ml were treated with the antibody (1:200 final dilution) according to the procedure described above. Stimulation with phytohemagglutinin (PHA) after anti-Thy-1.2 plus complement treatment showed a reduction of the mitogenic response by ~91%. (The monoclonal anti-Ly-2.2 antibody and the monoclonal anti-Thy-1.2 antibody were a gift of Dr. U. Hämmerling, Sloan-Kettering Institute for Cancer Research.) The elimination of adherent macrophages and B cells was carried out by passing spleen cells over a nylon-wool column according to the method of Julius et al. (12). After elimination procedures, the cell numbers were readjusted to standard concentrations.

In Vitro Cultures. 5 × 10^5 responder lymphocytes were cultivated in flat-bottomed microtiter plates (Fisher Scientific Co., Pittsburgh, Pa.) with either 5 × 10^5 irradiated spleen cells (2,000 rad) or 2 × 10^5 testicular cells as stimulators in a total vol of 200 μl. The plates were kept in a humidified atmosphere of 7% CO_2 in air in a 37°C incubator. After 4 d of cultivation, 20 μl of [^{3}H]thymidine solution (50 μCi/ml [^{3}H]thymidine; 0.5 μmol cold thymidine in PBS) was added; 16 h later, the labeled cultures were harvested in a Titertek multiple cell harvester (Flow Laboratories, Inc., Rockville, Md.) and the radioactivity was determined in a liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The mean values and the standard deviations were estimated from five replicate cultures.

For mitogenic stimulation, 2 × 10^5 spleen cells/well were cultivated in presence of 0.1 mitogenic U of PHA (Burroughs-Wellcome & Co., Research Triangle Park, N. C.) in a total vol of 200 μl for 3 d, labeled with [^{3}H]thymidine, and harvested 4 h later.

The stimulation index was determined by the following calculation:

$$\frac{\text{counts per minute of the mean of the experimental group}}{\text{counts per minute of the mean of the background}}.$$

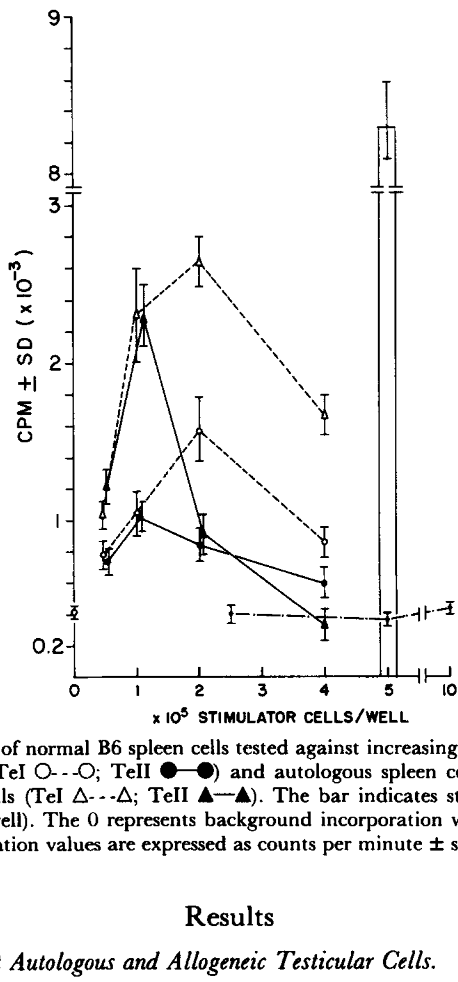

FIG. 1. Dose-response of normal B6 spleen cells tested against increasing concentrations of autologous testicular cells (TeI ○- - -○; TeII ●—●) and autologous spleen cells ●-·-●, and against allogeneic testicular cells (TeI △- - -△; TeII ▲—▲). The bar indicates stimulation by allogeneic spleen cells (5 × 10^5/well). The 0 represents background incorporation without stimulators. The ^{3}H-thymidine incorporation values are expressed as counts per minute ± standard deviations.

Results

Dose-Response against Autologous and Allogeneic Testicular Cells. We were interested in lymphocyte reactivity against the immunologically privileged testicular germ cells, on the assumption that they would provide an interesting model for studying immune reactions against autologous antigens. To obtain these cells, testis cells were fractionated into two subpopulations: (*a*) fraction TeII, that contained mostly germ cells and Sertoli cells, and (*b*) fraction TeI, that contained an enriched population of Leydig and other interstitial cells and some contaminating germ cells. We intended to concentrate preferentially on germ cells and to use the TeI fraction as the most appropriate controls.

The effect of both fractions of autologous and allogeneic testicular cells was tested on splenic lymphocyte proliferation in vitro and determined by [^{3}H]thymidine uptake after 5 d of cultivation. The proliferative response against autologous TeII fraction is shown in Fig. 1. Lymphocyte reactivity against this fraction was very low. No significant lymphocyte proliferation over background level was observed at high stimulator-cell concentrations (4 × 10^5 TeII cells/microtiter well). Only with decreasing cell concentrations did lymphocyte proliferation occur. The peak of the stimulation was reached with 1 × 10^5 autologous TeII cells per well, indicating a stimulation index of 2.4. A similar reaction pattern was observed with allogeneic TeII stimulators, except that the stimulation peak with 1×10^5 cells per well was higher than with autologous TeII stimulators (stimulation index: 5.4).

In experiments testing lymphocyte reactivity against the TeI fraction, we found that autologous TeI cells did induce stimulation in the responder cells (Fig. 1). This phenomenon is limited to testicular cells because autologous irradiated spleen cells failed to induce lymphocyte proliferation. The reactivity against autologous TeI cells was dose dependent; the peak of the response was reached with 2×10^5 TeI cells per well with a stimulation index of 3.6. In contrast to autologous TeII cells, stimulation against the TeI fraction was 46% higher at this cell concentration ($P < 0.01$). With increasing stimulator cell concentrations (4×10^5 cells per well) lymphocyte proliferation decreased. This might be a result of contaminating germ cells derived from broken tubules during the cell preparation that may have inhibitory effects on lymphocyte proliferation. In addition to thymidine uptake, stimulation was monitored by the counting of blasts. Approximately 10% more blasts were detected in cultures stimulated at the optimal TeI concentration than in unstimulated controls.

As expected, allogeneic TeI cells did stimulate lymphocyte proliferation. The reactivity against these cells was similar to the activity against autologous TeI cells in terms of the peak of the response and the decrease with higher stimulator cell concentration. The peak of the stimulation was reached with 2×10^5 cells per well (stimulation index: 6.1); at this concentration the reactivity was ~66% higher than against allogeneic TeII cells ($P < 0.01$). However, alloreactivity against TeI cells was much lower than against spleen cells (Fig. 1).

It has been shown that autoantigens are sometimes revealed by treatment with various kinds of proteases (13). We tested for this possibility by measuring lymphocyte reactivity to mechanically fractionated testicular cells compared to protease-treated stimulators. Table I shows that testicular cells released by either mechanical treatment or protease treatment induce comparable effects. The higher stimulation against protease-treated testicular cells, observed in some experiments, was probably a result of the better viability of cells prepared by this more gentle treatment.

Suppression of Autologous and Allogeneic Immune Response with Autologous Testicular Cells. The unexpected low reactivity of spleen cells against the autologous sequestered testicular cell fraction could obviously result either from a failure of TeII cells to stimulate or from their ability to induce active suppression. The second possibility seemed the more likely, because the proliferative response increased with decreasing cell concentration. To test this, autologous TeII cells were cocultivated with stimulators known to be capable of inducing lymphocyte proliferation; e.g., autologous and allogeneic TeI cells, and allogeneic spleen cells. The results are depicted in Tables II and III. The response against autologous and allogeneic TeI cells was significantly decreased in the presence of autologous TeII cells, with lymphocyte proliferation in both cases reduced by 64% ($P < 0.01$) (Table II). It can be excluded that cell density effects caused the reduction of the responder cell proliferation, doubling the stimulator concentration by coculturing of TeI plus TeII cells (final stimulator concentration = 4×10^5 cells/well). With the same cell concentration (4×10^5 cells/well) proliferation against autologous TeI cells is 55% and against allogeneic TeI cells is 63% higher. Stimulation against allogeneic spleen cells was even more strongly depressed, to as little as 10% of controls (Table III). The suppressive effect of autologous TeII cells was clearly dependent on the cell concentration. Autologous TeI cells did not affect allogeneic spleen cell responses (Table III).

TABLE I
Stimulation against Protease-treated or Mechanically Treated Testicular Cells

Experiment	Responders	Stimulators*	Protease treated		Mechanically treated	
			cpm ± SD	SI‡	cpm ± SD	SI
1	B6 spleen cells	—	575 ± 109		463 ± 134	
	B6 spleen cells	B6 TeI	2,181 ± 536	3.7	1,006 ± 96	2.1
	B6 spleen cells	B6 TeII	812 ± 89	1.4	446 ± 108	0.9
	B6 spleen cells	A/J TeI	1,245 ± 302	2.1	1,723 ± 391	3.7
	B6 spleen cells	A/J TeII	534 ± 212	0.9	364 ± 51	0.8
2	A/J spleen cells	—	401 ± 21		488 ± 71	
	A/J spleen cells	A/J TeI	1,542 ± 116	3.8	1,344 ± 158	2.7
	A/J spleen cells	A/J TeII	500 ± 134	1.2	233 ± 86	0.5
	A/J spleen cells	B6 TeI	2,084 ± 356	5.1	1,373 ± 179	2.8
	A/J spleen cells	B6 TeII	938 ± 375	2.3	257 ± 84	0.5

* 2×10^5 stimulators/well.
‡ SI, stimulation index.

TABLE II
Cocultivation of Autologous and Allogeneic TeI Cells with Autologous TeII Cells

Responders	Stimulators	cpm ± SD	SI
B6 spleen cells	—	615 ± 35	
B6 spleen cells	B6 spleen cells*	653 ± 70	1.07
B6 spleen cells	B6 TeI (4×10^5)	1,562 ± 141	2.53
B6 spleen cells	B6 TeI (2×10^5)	1,896 ± 270	3.08
B6 spleen cells	B6 TeII (2×10^5)	993 ± 160	1.61
B6 spleen cells	B6 TeI (2×10^5) + B6 TeII (2×10^5)	700 ± 52	1.13
B6 spleen cells	CBA spleen cells*	9,366 ± 476	15.22
B6 spleen cells	CBA TeI (4×10^5)	2,239 ± 234	3.64
B6 spleen cells	CBA TeI (2×10^5)	2,260 ± 464	3.67
B6 spleen cells	CBA TeII (2×10^5)	677 ± 131	1.10
B6 spleen cells	CBA TeI (2×10^5) + B6 TeII (2×10^5)	832 ± 42	1.35

* 5×10^5 spleen cells/well.

Nature of the Responder Cells. As shown in Tables II and III, lymphocyte proliferation is suppressed in the presence of autologous and allogeneic TeII cells. To characterize the lymphoid cell type responsible for the suppression associated with autologous TeII cells, physical and serological separation methods were used. Table IV summarizes the response of spleen cells depleted of B cells and adherent macrophages by passage over a nylon-wool column. Reactivity against autologous and allogeneic TeII cells is not changed significantly, which suggests that macrophages can be excluded as significant inhibitors of lymphocyte proliferation (14).

In a further analysis of the origin of suppressor activity, spleen cells were pretreated with anti-Ly-2.2 antibody plus complement to eliminate suppressor T cells. Table V

TABLE III

Cocultivation of Autologous TeI and TeII Cells with Allogeneic Spleen Cells

Responders	Stimulators	cpm ± SD	SI
B6 spleen cells	—	364 ± 136	
B6 spleen cells	B6 spleen cells	454 ± 157	1.2
B6 spleen cells	A/J spleen cells	13,500 ± 509	37.0
B6 spleen cells	B6 TeI (4 × 10^5)	1,026 ± 88	2.8
B6 spleen cells	B6 TeI (2 × 10^5)	1,602 ± 101	4.4
B6 spleen cells	A/J spleen cells + B6 TeI (4 × 10^5)	14,725 ± 1,392	40.4
B6 spleen cells	A/J spleen cells + B6 TeI (2 × 10^5)	14,677 ± 669	40.3
B6 spleen cells	B6 TeII (4 × 10^5)	460 ± 64	1.2
B6 spleen cells	B6 TeII (2 × 10^5)	620 ± 58	1.7
B6 spleen cells	B6 TeII (1 × 10^5)	1,228 ± 241	3.3
B6 spleen cells	B6 TeII (0.5 × 10^5)	591 ± 95	1.6
B6 spleen cells	A/J spleen cells + B6 TeII (4 × 10^5)	949 ± 113	2.6
B6 spleen cells	A/J spleen cells + B6 TeII (2 × 10^5)	1,240 ± 151	3.4
B6 spleen cells	A/J spleen cells + B6 TeII (1 × 10^5)	12,799 ± 1,113	35.7
B6 spleen cells	A/J spleen cells + B6 TeII (0.5 × 10^5)	11,174 ± 1,058	30.6

TABLE IV

Enrichment of T Cells by Nylon-Wool Passage

Responders	Stimulators	Untreated		T cell enriched	
		cpm ± SD	SI	cpm ± SD	SI
B6 spleen cells	—	426 ± 43		553 ± 17	
B6 spleen cells	B6 TeI	1,563 ± 385	3.6	2,640 ± 586	4.8
B6 spleen cells	B6 TeII	640 ± 156	1.5	765 ± 24	1.3
B6 spleen cells	AKR spleen cells	6,390 ± 360	15.0	9,936 ± 226	17.9
B6 spleen cells	AKR TeI	4,188 ± 450	9.8	7,242 ± 514	13.0
B6 spleen cells	AKR TeII	2,109 ± 388	4.9	3,201 ± 558	5.8

shows that such elimination results in a normal allogeneic spleen cell response, as well as reactivity against autologous TeI cells. Moreover, the reactivity against autologous TeII cells alone reached the same level as the response against TeI cells. These data indicate that immunosuppression in the presence of autologous TeII cells is induced by suppressor T cells.

Also demonstrated in Table IV is the proliferative response against autologous and allogeneic TeI cells after depletion of B cells and adherent macrophages. Because the reactivity is still maintained, B cells are unlikely to be involved in response. To define

TABLE V

Pretreatment of Responder Cells with Ly-2.2 Antiserum + Complement

Responders	Stimulators	Untreated		Anti-Ly-2.2 + complement-treated	
		cpm ± SD	SI	cpm ± SD	SI
B6 spleen cells	—	1,710 ± 68		1,102 ± 644	
B6 spleen cells	B6 TeI	6,217 ± 563	3.63	8,797 ± 579	7.98
B6 spleen cells	B6 TeII	3,023 ± 708	1.76	12,511 ± 1,524	11.35
B6 spleen cells	B6 TeI + B6 TeII	1,245 ± 229	0.72	7,171 ± 640	6.50
B6 spleen cells	BALB/c spleen cells	19,585 ± 1,845	11.45	20,459 ± 1,766	18.56
B6 spleen cells	BALB/c spleen cells + B6 TeII	6,052 ± 727	3.53	19,497 ± 1,900	17.69

TABLE VI

Pretreatment of Responder Cells with Thy-1.2 Antiserum + Complement

Responders	Stimulators	Untreated		Anti-Thy-1.2 + complement-treated	
		cpm ± SD	SI	cpm ± SD	SI
B6 spleen cells	—	770 ± 84		1,008 ± 154	
B6 spleen cells	B6 TeI	4,532 ± 1,169	5.88	1,808 ± 564	1.80
B6 spleen cells	B6 TeII	840 ± 131	1.09	2,040 ± 361	2.04
B6 spleen cells	A/J spleen cells	34,052 ± 10,210	44.2	1,167 ± 255	1.16
B6 spleen cells	A/J TeI	14,143 ± 2,576	18.2	7,695 ± 1,031	7.69
B6 spleen cells	A/J TeII	2,517 ± 1,357	3.2	859 ± 454	0.85
B6 spleen cells	PHA	30,096 ± 3,516	39.08	2,003 ± 427	2.00

whether T cells were proliferating, spleen cells were pretreated with monoclonal anti-Thy-1.2 antibody plus complement. Table VI shows that elimination of T cells significantly reduced stimulation ($P < 0.01$) but does not abolish it completely. As expected, the response against allogeneic spleen cells is totally abrogated. Likewise, the mitogenic response against PHA is reduced by 91%. This data indicate that, besides T cells, other lymphoid cells respond on stimulation by fraction TeI cells.

Nature of the Testicular Cell Population Inducing Suppressor T Cell Activity. Further experiments were undertaken to confirm that the cells responsible for suppressor T cell induction were, in fact, germ cells within the TeII fraction. We took advantage of the fact that mutations at the W and W^v locus produce heterozygotes that are sterile. W/W^v compound males have normally developed somatic components of the testis but almost no germ cells (15). Thus, in these males the TeI population is comparable to that of wild-type animals, but the TeII fraction contains essentially only Sertoli cells. The responder lymphocytes were derived from wild-type siblings of F_1 W/W^v animals. Table VII shows that syngeneic TeI and TeII cells of W/W^v mice evoked a strong lymphocyte stimulation which even exceeded the reactivity against allogeneic spleen cells. Moreover, W/W^v TeII cells did not suppress the response against allogeneic spleen cells.

TABLE VII

Stimulation against Germ-Cell-depleted Testicular Cells

Responders	Stimulators	cpm ± SD	SI
(+/+) spleen cells	—	550 ± 53	
(+/+) spleen cells	(+/+) TeI	3,668 ± 440	6.66
(+/+) spleen cells	(+/+) TeII	1,533 ± 255	2.78
(+/+) spleen cells	W/W^v spleen cells	572 ± 55	1.04
(+/+) spleen cells	W/W^v TeI	16,963 ± 1,093	30.84
(+/+) spleen cells	W/W^v TeII	16,450 ± 2,993	29.90
(+/+) spleen cells	B6 H-2^k spleen cells	13,528 ± 1,664	24.10
(+/+) spleen cells	B6 H-2^k TeI	5,627 ± 128	10.23
(+/+) spleen cells	B6 H-2^k TeII	2,670 ± 420	4.85
(+/+) spleen cells	B6 H-2^k spleen cells + (+/+) TeII	1,598 ± 172	2.90
(+/+) spleen cells	B6 H-2^k spleen cells + W/W^v TeI	13,618 ± 2,822	24.76
(+/+) spleen cells	B6 H-2^k spleen cells + W/W^v TeII	13,270 ± 2,806	24.2

Discussion

These experiments describe lymphocyte reactivity in vitro against autologous and allogeneic testicular cells in the presence of autologous serum. We investigated lymphocyte reactivity against autoantigens on testicular cells derived from the seminiferous tubules that normally are not accessible to the immune system, and thus should be expected to be autoantigenic. This cell population contained Sertoli cells and germ cells (TeII). As controls, we tested reactivity against cells deriving from non-immunologically privileged sites in the testis, a fraction containing Leydig and other interstitial cells (TeI). These experiments revealed two contrary and unexpected findings: (*a*) somatic cells of the testes deriving from nonprivileged sites stimulated lymphocyte proliferation; (*b*) germ cells deriving from the immunologically privileged site suppressed lymphocyte proliferation.

The reactive lymphocytes were primarily T cells. The proliferative response to TeI cells was not altered by elimination of B cells and adherent macrophages from responder populations, whereas stimulation was considerably reduced when only T cells were removed by complement-dependent lysis with anti-Thy-1.2 serum. However, lymphocyte reactivity was not completely abolished in this case, indicating that part of the responder population was resistant to anti-Thy-1.2 plus complement treatment. Several explanations for this observation are possible. It is likely that treatment with anti-Thy-1.2 antibody plus complement generally depletes, but does not eliminate, the entire T cell population. It may also be that the specific types of T cells that react against testicular cells express relatively low concentrations of Thy-1.2 antigen and are, thus, relatively insensitive to lysis. It has been shown in fact that Ly-$1,2,3^+$ cells, which are precursors of helper and suppressor T cells, are resistant to a single treatment with anti-Thy-1.2 plus complement (16); it is thus possible that such cells escaped elimination and thus differentiated to Ly-1^+ cells (helper-T cells) during the 5 d of in vitro incubation. Furthermore, it cannot be excluded that another lymphoid cell population is involved, such as natural-killer cells (17).

The interstitial cell population of normal mice (TeI) provided significant stimulation, and populations of syngeneic somatic cells, interstitial cells or Sertoli cells from germ-cell depleted W/W^v mice, produced several fold stronger proliferation of the responding lymphocytes. Also, TeII fractions that contained germ cells stimulated lymphocyte proliferation after elimination of suppressor T cells (see below). Theoretically there are several possibilities for lymphocyte stimulation. Collagenase and trypsin were used to prepare the two testicular cell fractions, and, therefore, protease treatment could have exposed normally hidden autoantigen or modified self antigens (5, 6, 13). However, mechanically prepared testicular cells induced comparable lymphocyte proliferation. Thus, the probability of exposure or artificial alterations of cell-surface antigens seems rather unlikely. Modulation of the immune response by sex hormones has been reported by various authors (18–20), and because Leydig cells produce testosterone, the question arose whether the stimulation against cells of TeI fraction was elicited by secreted hormone in the culture supernate, or by hormonal modification of self antigen on the testicular cells themselves. The observation that androgen decreases autoimmune reactions in NZB/W mice (21), and thus is immunosuppressive, makes this possibility less likely. Another possible explanation is the expression of viral antigens on testicular cells. Various reports demonstrate the spontaneous appearance of endogenous virus products after in vitro cultivation (22–24). Furthermore, immunofluorescence techniques have revealed gp70 in the epithelium of the epididymis and vas deferens, with quantitative differences in various mouse strains (25). However, gp70 has never been identified in the testis. These observations, in addition to our finding that autologous spleen cell stimulators are incapable of inducing proliferation, lower the possibility of viral infection as the cause of the antigenicity of autologous TeI cells, but certainly does not exclude this.

The antigenic determinants responsible for lymphocyte stimulation against autologous testicular cells are not yet identified. Recently, the recognition of self major histocompatibility complex (MHC) antigens has been shown to be essential for the response against foreign antigens. This has been demonstrated in T cell responses against chemically modified cells (26), virus-infected target cells (27), weak histocompatibility antigens (28), and male-specific H-Y antigen (29). Moreover, the immunological memory and specificity of rat lymphocytes against syngeneic (somatic) testicular cells which has been demonstrated in vitro, was strongly restricted to self MHC antigens, and to tissue-specific antigens (30). The Ia antigens of the H-2 system are known to have a restricted tissue distribution (31). Although the presence of Ia antigens on testicular somatic cells has not been shown so far, it has been demonstrated on spermatocytes (32) and sperm (31). Thus, it can be suggested that stimulation against autologous testicular cells is caused by recognition of self MHC antigen(s) and testicular-cell antigen(s). Questions concerning the specificity of the stimulation are now under study.

The lymphocyte proliferation induced by autologous somatic testicular cells contrasts strongly with the suppression induced by germ cells of the same animals. This immune suppression can be abrogated by anti-Ly-2.2 plus complement treatment of the responder cells and thus is a result of the activation of suppressor T cells. The target activity of the suppressor cells seems to be nonspecific, because they are capable of inhibiting lymphocyte proliferation against syngeneic TeI cells as well as against allogeneic spleen cells. The degree of stimulation or suppression apparently depends very delicately on the relative proportions of germ cells in the two testicular cell fractions; that is, on the number of cells in each population that are able to induce suppressor T cell activity. Increasing concentrations of TeI cells lead to a decrease in stimulation, presumably because the number of contaminating germ cells is increased, whereas low concentrations of TeII cells or pure Sertoli cells (fraction TeII of W/W^v mice) resulted in an increased proliferative response.

The determinants on the germ cells responsible for the induction of suppressor cells have not been identified either. However, it has been shown that germ cells express embryonic antigens (33), and furthermore, many reports demonstrate suppressor functions of embryonic cells or embryonic antigens; e.g., embryonic hepatocytes suppress graft-versus-host and mixed-leukocyte responses (34), and human or murine α-fetoprotein regulates the induction of suppressor cells (35, 36).

Our observations suggest that germ cells have similar immunoregulatory functions which may operate via embryonic antigens. Under normal in vivo conditions, germ cells are segregated from the body by the blood-testis barrier which preserves the microenvironment of the developing spermatozoa and ensures their isolation (37). In the event the blood-testis barrier is inoperative; e.g., by physical injury or inflammation, germ cells may prevent autoimmune reactions by the induction of suppressor T cells which generate immunological protection. Similarly, embryonic antigens expressed on early mouse embryos (38) may be responsible for immunological protection of the fetus from the maternal immune system in early stages of pregnancy.

Summary

We have investigated the regulation of self tolerance in mice by examining lymphocyte reactivity in vitro against two subpopulations of autologous testicular cells: germ cells that were derived from the seminiferous tubules, and interstitial somatic cells. In the presence of germ cells, lymphocyte proliferation was strongly reduced. In contrast, somatic interstitial cells stimulated lymphocyte proliferation. In both cases, reactive lymphocytes were mostly T cells. Suppressor T cells activated by autologous germ cells were nonspecific and capable of inhibiting lymphocyte proliferation against autologous and allogeneic somatic testicular cells as well as against allogeneic spleen cells. Suppression was abrogated after treatment of the responder lymphocytes with anti-Ly-2.2 serum plus complement. Lymphocyte proliferation by autologous interstitial cells was considerably reduced, but not completely abolished, by complement-dependent lysis with anti-Thy-1.2 serum. This may indicate the participation in proliferation of a lymphoid cell population other than T cells.

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