Immunology 1983 **49** 191

The role of I-J in the suppressor T-cell circuit which influences the effector stage of contact sensitivity: antigen together with syngeneic I-J region determinants induces and activates T suppressor cells

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Accepted for publication 26 November 1982

Summary. One of the T suppressor circuits induced by picrylsulphonic acid includes the T suppressor cell (Ts-eff) which acts at the efferent stage of the contact sensitivity reaction and produces antigen-specific T suppressor factor (TsF). This factor does not act directly but arms a T acceptor cell (Tacc). This Tacc liberates a non-specific inhibitor when it is armed with TsF and then exposed to picrylated cells sharing the I-J genotype of the source of the TsF. This paper investigates the role of I-J region gene products in this T suppressor circuit. Two approaches were used. Syngeneic CBA (H-2^k) lymphocytes were separated into I-J^+ and I-J^- cells by treatment with anti-I-J^k serum followed by panning on anti-immunoglobulin plates. The cells were then picrylated and used as a source of antigen. Alternatively, B10.A congeneic mice syngeneic (5R) or allogeneic (3R) with CBA at the I-J locus were picrylated and used similarly. The main findings were as follows.

(i) The intravenous injection of picrylated I-J^+ spleen cells but not a similar number of I-J^- cells induced Ts-eff which blocked the transfer of contact sensitivity. Picrylated unseparated cells syngeneic, but not allogeneic, at the I-J locus were also effective.

(ii) It is known that the lymphocytes of mice injected with picrylsulphonic acid and then re-exposed to antigen by painting with picryl chloride liberate TsF *in vitro*. The re-exposure to antigen can be replaced by the intravenous injection of picrylated I-J^+ cells or by cells syngeneic at the I-J locus the day before harvesting the spleen cells.

(iii) The release of non-specific inhibitor by Tacc armed with TsF requires exposure to picrylated I-J^+ cells or cells syngeneic at the I-J locus. The requirement for antigen on a cell bearing syngeneic I-J suggests that antigen together with I-J is an activation signal in this T-cell circuit. The simplest explanation is that the receptor of the pristine Ts and of the mature Ts-eff is similar to T suppressor factor.

INTRODUCTION

Several workers have proposed that the presentation of antigen on I-J^+ cells is the induction signal for T suppressor cells (Mitchison, 1981). However the evidence is based on systems using transplantation antigens associated with allogeneic I-J and its relevance to the induction of T suppressor cells to other antigens is unclear. The role of I-J as an induction signal is also suggested by the fact that the suppressor

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0019-2805/83/0500-0191$02.00

inducer cell, which probably gives rise to suppressor cells by a process of immunization, is I-J$^+$ in the system of antibody response to sheep red blood cells (Eardley *et al.*, 1980). Moreover, in delayed hypersensitivity to nitrophenylacetyl (NP), the Ts_1, which is I-J$^+$ and bears idiotype, induces the anti-idiotypic suppressor Ts_2 by a process of immunization (Okuda *et al.*, 1981a).

Recently Zembala *et al.* (1982a) studied the T suppressor circuit which includes the T suppressor cell which acts at the efferent (expression) stage of the contact sensitivity reaction (Ts-eff), antigen-specific T suppressor factor (TsF) and the T acceptor cell (Tacc) (see Fig. 1). In this circuit the Ts-eff, which probably corresponds to the Ts_3 in the NP system, does not inhibit contact sensitivity directly. In fact it arms an antigen-non-specific T acceptor cell. This cell, when armed and subsequently exposed to antigen, e.g. picrylated or oxazolonated spleen cells, releases a non-specific inhibitor of the transfer of contact sensitivity. However the release of the non-specific inhibitor only occurs when the antigen is presented on cells which are syngeneic in the I-J region with the cells producing the TsF (Zembala, Asherson & Colizzi, 1982b).

This system can be dissected into three stages: the induction of the Ts-eff; the release of antigen-specific TsF from the Ts-eff and the arming of the Tacc; and finally the release of non-specific inhibitor by the armed Tacc. Antigen is needed at each of these stages and can be provided by haptenized spleen cells. Moreover the effect of the genotype of the haptenized spleen cell can be investigated in two ways. Syngeneic cells can be separated into I-J$^+$ and I-J$^-$ cells using conventional anti-I-J sera and then haptenized and tested. Alternatively the activity of haptenized spleen cells from strains of mice with different I-J genotypes can be studied. Using these two approaches, this paper shows that antigen on syngeneic I-J$^+$ cells is an induction signal for this T suppressor circuit and is required for the induction of the Ts-eff, the release of antigen-specific TsF by the Ts-eff and the release of non-specific inhibitor by Tacc armed with TsF.

MATERIALS AND METHODS

The general methods are given in Zembala *et al.* (1982a,b).

Mice

CBA, BALB/c and C57BL/10 were bred locally. Other mice were obtained from OLAC.

Preparation of picrylated spleen cells ('antigen-presenting cells')

The picrylated cells used as a source of antigen were prepared by depleting normal spleen cells of red blood cells with Boyle's solution and haptenizing with 1 mM neutralized picrylsulphonic acid (PSA) in Dulbecco's phosphate-buffered saline for 10 min at room temperature. The cells were then washed four times.

To obtain purified I-J$^+$ and I-J$^-$ cells, normal nylon T spleen-cells were incubated with anti-I-J serum (10^8

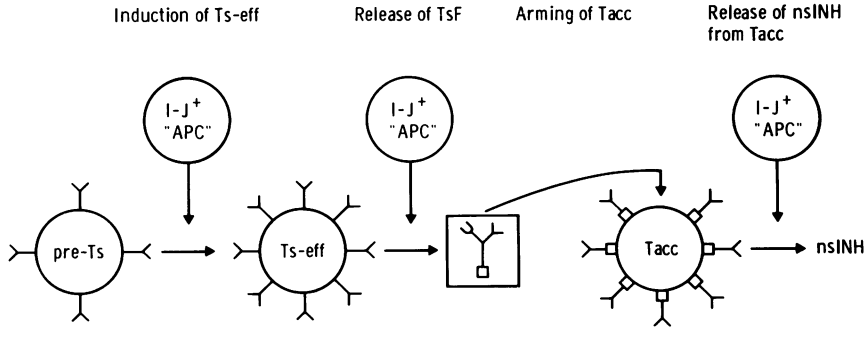

Figure 1. The diagram illustrates the hypothesis that there are three stages at which the picrylated I-J$^+$ "antigen presenting cell" ("APC") affects the T suppressor circuit. At the induction stage it causes the precursor T suppressor cell (pre-Ts) to differentiate into a T suppressor effector cell (Ts-eff). It then causes the Tc-eff to release antigen-specific T suppressor factor (TsF). The TsF arms the T acceptor cell (Tacc) which is then triggered by the picrylated "antigen presenting cell" to release non-specific inhibitor (nsINH) of the transfer of contact sensitivity. The receptor and TsF on the cells are shown diagrammatically and it is suggested that the TsF resembles the receptor on the precursor T suppressor cell and on the Ts-eff. The box illustrates a model of the TsF in greater detail and shows its binding site(s) for the T acceptor cell, the picryl antigen and for the I- gene product.

cells in 1 ml 1/26 dilution of anti-I-J 3R anti-5R serum) at 4° for 1 hr (Malkovsky *et al.*, 1982). After washing, the cells were panned on 9-cm bacteriological Petri dishes coated with $F(ab')_2$ rabbit anti-mouse immunoglobulin. After 1 hr the non-adherent I-J$^-$ cells were poured off and the adherent I-J$^+$ cells were recovered by rubbing (Zembala *et al.*, 1982a). The cells were then haptenized with 1 mM PSA, and then injected into groups of five recipients. Viable cell counts assessed by trypan blue dye exclusion are used throughout.

Production and detection of Ts-eff

Mice were injected intravenously on day 0, 3 with 3·5 mg and 3 mg PSA or with picrylated spleen cells. Three days after the last injection of PSA or five days after the injection of cells, the spleens were collected. The presence of Ts-eff was tested in a passive transfer experiment by mixing 5×10^7 spleen and lymph-node cells with 5×10^7 4-day picryl immune cells. The cells (10^8) were immediately injected into each of five mice. These were challenged on the ears with 1% picryl chloride in olive oil. Contact sensitivity was assessed by the increment of ear thickness at 24 hr in units of 10^{-3} cm $\pm$ standard deviation. The percentage depression of contact sensitivity was given by the means of the following groups in the formula $100 \times$ (positive − experimental)/(positive − negative).

Production and detection of T suppressor factor (TsF)

Mice injected with PSA on day 0 and 3 were painted with 5% ethanolic picryl chloride (0·15 ml) or injected with picrylated cells to provide a second exposure to antigen on day 6. The spleen cells were taken one day later and cultured (10^7 ml^{-1}, 48 hr, 37°) in RPMI 1640 with added glutamine, penicillin and streptomycin and 5% inactivated foetal calf serum. In some experiments mice were first injected with picrylated cells and then painted 6 days later with picryl chloride.

The TsF was assayed in a two step assay. Briefly, 4-day oxazolone immune, nylon wool passed, T cells were used as a source of Tacc. They were armed ($1{\cdot}5 \times 10^8$) with presumptive TsF (5 ml), diluted and washed once. The cells (5×10^7) were then incubated with picrylated spleen cells, centrifuged and resuspended in medium (5 ml) with 2·5% inactivated foetal calf serum. The 2-hr supernatant contained non-specific inhibitor. This was assayed by its ability to inhibit the passive transfer of contact sensitivity to oxazolone using groups of five mice. In practice, pooled lymph-node and spleen cells ($2{\cdot}5 \times 10^8$) from 4-day immune mice were incubated in 5 ml of non-specific inhibitor (45 min, 37°). Cells ($4{\cdot}5 \times 10^7$) were injected into each mouse.

Treatment with anti-Thy-1.2

Spleen cells were treated with 1/1000 monoclonal anti-Thy-1.2 antibody (OLAC) at 5×10^7/ml for 30 min at room temperature. The cells were diluted, spun down and then exposed to 25% rabbit complement (selected so as to be non-toxic) for 45 min at 37°. As a control, cells were treated with rabbit complement only. No adjustment was made for cell losses.

Statistics

Student's double-tailed *t* test was used.

RESULTS

I-J$^+$ cells needed for the induction of Ts-eff

Mice were injected with picrylated I-J$^+$ T cells, prepared by coating with anti-I-J serum and panning on anti-immunoglobulin plates. Other mice were injected with picrylated I-J$^-$ T cells. Five days later the spleen cells of the recipients were tested for the presence of Ts-eff, which were assayed by their ability to inhibit the passive transfer of contact sensitivity. Table 1, Exp. 1 shows that 7×10^6 picrylated I-J$^+$ cells induced Ts-eff, while the same number of picrylated I-J$^-$ cells were inactive. The activity of larger number of 'I-J$^-$' cells may have been due to residual I-J$^+$ cells. These findings were confirmed in Exp.2.

The role of the I-J genotype was investigated by injecting picrylated spleen cells from congeneic B10.A mice into CBA mice. Table 2 shows that B10.A(5R), which only share I-J^k and I-E^k with CBA mice, induced Ts-eff, while 3R mice, which only share I-E^k, were inactive. In keeping with this, 4R mice, which share K^k and I-A^k, and C3H.OH, which share D^k, were also ineffective. The legend to Table 2 confirms that the suppressor cells were Thy-1.2 positive. Taken together these results indicate that I-J$^+$ cells are required for the induction of Ts-eff.

The following experiment shows that the Ts-eff produced by injecting picrylated cells and those produced by the conventional procedure of injecting picrylsulphonic acid liberate TsF. TsF was measured by the indirect acceptor cell assay based on arming Tacc with TsF, triggering the release of non-specific inhibitor with picrylated cells and finally assaying the non-specific inhibitor by the inhibition of the passive

Table 1. Induction of T suppressor cells (Ts-eff) by the intravenous injection of picrylated I-J$^+$ and I-J$^-$ normal spleen cells

Cells injected		Assay for Ts-eff			
		Exp. 1		Exp. 2	
Positive assay control		5·6±0·41		4·7±0·28	
Negative assay control		2·9±0·96		3·0±0·35	
Picrylated spleen cells	5×10^7	5·8±0·54	7%		
Picrylated I-J$^+$ cells	7×10^6	3·7±0·27	71%*	3·0±0·41	100%*
Picrylated I-J$^-$ cells	7×10^6	5·8±0·85	0%	4·6±1.25	6%
Picrylated I-J$^-$ cells	5×10^7	4·8±0·28	30%	3·1±0·55	94%*

Mice were injected with picrylated I-J$^+$ or I-J$^-$ spleen cells. Five days later their spleens were assayed for the presence of Ts-eff by their ability to inhibit passive transfer of contact sensitivity to picryl chloride. In Exp. 2, 10^7 picrylated I-J$^+$ and 10^7 and 3×10^7 I-J$^-$ cells were injected. The figures show contact sensitivity at 24 hr in units of 10^{-3} cm ± standard deviation. The percentages refer to the percent depression of contact sensitivity and provide a measure of Ts-eff activity.

* Significantly different from positive assay control $P<0.002$.

† Significantly different from positive assay control $P<0.02$.

Table 2. Induction of T suppressor cells (Ts-eff) by the intravenous injection of picrylated cells of various I-J genotypes into CBA mice

Cells injected	Major histocompatibility complex							Assay for Ts-eff			
	K	I-A	I-B	I-J	I-E	I-C	D	Exp. 1		Exp. 2	
Positive assay control								4·3±0·29		4·1±0·25	
Negative assay control								2·9±0·25		1·5±0·35	
Picrylated CBA cells	k	k	k	k	k	k	k	2·9±0·30	100%*		
B10.A(5R)	b	b	b	k	k	d	d	2·5±0·40	100%*	2·1±0·25	77%*
B10.A(3R)	b	b	b	b	k	d	d	4·3±0·64	0%		
B10.A(4R)	k	k	b	b	b	b	b			3·9±0·48	8%
C3H.OH	d	d	d	d	d	d	k	3·9±0·47	29%		

CBA mice were injected with 5×10^7 picrylated spleen cells. Five days later their spleens were assayed for the presence of Ts-eff. Note that only picrylated syngeneic CBA cells and B10.A(5R) cells which share I-J^k with CBA induce Ts-eff. The Ts-eff were sensitive to anti-Thy-1.2 antibody and complement. In Exp. 2 cells Ts-eff from mice injected with picrylated 5R cells resisted treatment with rabbit complement (2.6±0·65) but were inactivated by anti-Thy-1.2 serum and complement (3·8±0·28).

* Significantly different from positive control $P>0.001$.

transfer of contact sensitivity. Table 3, Exp. 1 shows that Ts-eff induced by picrylated syngeneic I-J$^+$ cells produced TsF when painted the day before harvesting. In contrast, the cells from mice injected with picrylated syngeneic I-J$^-$ cells or spleen cells of an I-J allogeneic genotype and then painted, did not produce TsF. In Exp. 2 mice were injected with picrylated cells but the painting was omitted. However some production of TsF was still observed.

Picrylated I-J$^+$ cells needed for the release of TsF

It is known that mice injected with picrylsulphonic acid release TsF *in vitro* when re-exposed to antigen by painting with picryl chloride. However Table 4 shows that painting with picryl chloride can be replaced by the intravenous injection of picrylated I-J$^+$, but not I-J$^-$ cells. In keeping with this, only allogeneic cells with the same I-J^k genotype as the recipient CBA mice caused the release of TsF.

Picrylated I-J cells needed for the release of non-specific inhibitor from T cells armed with TsF

T acceptor cells (H-2^d) were armed with anti-picryl TsF (H-2^k) and mixed with picrylated CBA (H-2^k) I-J$^+$ or I-J$^-$ cells. The supernatant was taken 2 hr later and tested for the presence of nonspecific inhibitor. Table 5 shows that picrylated I-J$^+$ but not I-J$^-$ cells triggered the release of non-specific inhibitor. The lower part of the table shows that there was an I-J genetic restriction between the TsF and the picrylated cell when B10.A(5R) and 3R mice were used, and that only cells syngeneic in the I-J region with the source of the TsF triggered the release of non-specificcc inhibitor.

Table 3. Production of T suppressor factor (TsF) by T suppressor cells (Ts-eff) induced by the intravenous injection of I-J$^+$ and I-J$^-$ cells and of cells of various I-J genotypes into CBA mice

Cells injected		Assay for TsF			
		Exp. 1		Exp. 2	
Positive assay control		5·8±0·50		6·1±1·08	
Negative assay control		3·6±1·02		2·8±1·15	
Picrylated I-J$^+$ cells	1×10^7	3·1±0·85	100%*		
Picrylated I-J$^-$ cells	1×10^7	6·2±0·65	0%		
Picrylated I-J$^-$ cells	3×10^7	6·5±1·08	0%		
Picrylated B10.A(5R)	5×10^7	4·2±0·63	72%†	4·7±0·57	42%‡
Picrylated B10.A(4R)	5×10^7			6·2±0·65	0%
Picrylated B10.A(3R)	5×10^7	5·9±0·63	0%		
C3H	5×10^7	5·9±1·18	0%		
B10.A(5R) specificity control				6·6±0·82	

Mice were injected with picrylated syngeneic I-J$^+$ or I-J$^-$ cells or with various allogeneic cells. Six days later the mice were painted with picryl chloride to cause the release of TsF. Spleen cells were taken on day 6 and cultured for 48 hr; the supernatant taken at 48 hr was assayed for TsF. TsF was measured by an indirect acceptor-cell assay and its presence indicated by the depression of the transfer of contact sensitivity to oxazolone. The B10.A(5R) specificity control refers to an assay in which the Tacc armed with anti-picryl TsF was exposed to oxazolonated cells instead of picrylated cells. It shows that the TsF has antigenic specificity. In the first experiment the mice were painted with picryl chloride 1 day before harvesting with a view to augmenting the production of TsF. This was omitted in the second experiment.

* Significantly different from positive control $P<0·001$.

†Significantly different from positive control $P<0·01$.

‡ Significantly different from positive control $P<0·05$.

Table 4. Production of T suppressor factor (TsF) from T suppressor cells (Ts-eff) induced by picrylsulphonic acid and caused to release TsF by the injection of picrylated cells intravenously

Cells injected		Assay for TsF Exp. 1		Assay for TsF Exp. 2	
Positive assay control		5·8±0·50		6·4±0·22	
Negative assay control		3·6±1·02		2·3±0·57	
Picrylated I-J$^+$	1×10^7	3·5±0·70	100%*		
Picrylated I-J$^-$	1×10^7	5·6±1·65	9%		
Picrylated I-J$^-$	3×10^7	5·6±1·25	9%		
Picrylated B10.A(5R)	5×10^7	3·6±1·25	100%*	3·7±0·84	66%*
Picrylated B10.A(3R)	5×10^7	5·8±0·50	0%	6·0±0·61	10%
Picrylated C3H.OH	5×10^7	6·0±0·58	0%	6·0±7·10	10%
Picrylated CBA	5×10^7			3·5±0·50	71%*

Mice were injected with picrylsulphonic acid on days 0 and 3 to induce TsF. On day 6 the mice were injected with picrylated cells and the spleen cells were taken and cultured on day 7. The supernatant taken at 48 h was assayed for TsF. The indirect acceptor cell assay was used. As a control on the assay, TsF, made by injection of PSA followed by painting with picryl chloride, was used. (Exp. 1: 3·1±0·85, 100% inhibition. Exp. 2: 3·3±0·45, 76% inhibition.)

* Significantly different from positive control $P<0.02$.

Table 5. Triggering of armed T acceptor cells (Tacc) by incubation with I-J$^+$ and I-J$^-$ cells and cells of various I-J genotypes

Tacc	TsF	Picrylated spleen cell		Assay for non-specific inhibitor	
Positive control				6·0±0·87	
Negative control				3·6±0·55	
Ox BALB/c	Pic CBA	I-J$^+$ CBA	1×10^7	3·7±1·09	96%†
Ox BALB/c	Pic CBA	I-J$^-$ CBA	1×10^7	5·7±0·48	12%
Ox BALB/c	Pic CBA	I-J$^-$ CBA	3×10^7	4·7±0·57	54%‡
Ox BALB/c	Pic CBA	B10.A(5R)	3×10^7	3·6±0·42	100%*
Ox BALB/c	Pic CBA	B10.A(3R)	3×10^7	5·0±0·35	42%
Ox BALB/c	Pic CBA	C3H.OH	3×10^7	5·1±0·65	37%

Nylon T oxazolone immune T cells were used as T acceptor cells. They were armed with CBA anti-picryl TsF and then exposed to picrylated cells. After incubation for 2 hr, the supernatant was tested for the presence of non-specific inhibitor by the inhibition of passive transfer of contact sensitivity to oxazolone.

Significantly different from positive control *$P<0.001$, †$P<0.01$, ‡$P<0.05$.

DISCUSSION

The present experiments investigated the I-J genetic restriction in the T suppressor circuit which influences contact sensitivity. In this circuit the injection of picrylsulphonic acid induces the Ts-eff population which acts at the expression stage of the contact sensitivity reaction (see also Thomas, Watkins & Asherson, 1981). This population liberates antigen-specific TsF *in vitro*. This arms a T acceptor cell and this cell, when triggered with picrylated spleen cells which bear the same major histocompatibility complex genotype as the source of the TsF, liberates a non-specific inhibitor of contact sensitivity (see Fig. 1). This genetic restriction is located in the I-J sub-region and is due to the TsF and unrelated to the source of the Tacc (Zembala *et al.*, 1982b). The question was asked whether the same I-J genetic restriction was seen in the induction of Ts-eff by the intravenous injection of picrylated spleen cells and the production of antigen-specific T suppressor factor by Ts-eff.

The present results show that picrylated cells of syngeneic I-J genotype, but not of allogeneic I-J genotype, induce Ts-eff. The fact that allogeneic cells were ineffective suggested that the antigen was not reprocessed *in vivo* and then presented by the recipient's own cells. The role of I-J was confirmed by separating syngeneic cells into $I\text{-}J^+$ and $I\text{-}J^-$ using antisera and then picrylating. The Ts-eff were reassessed by their ability to block the passive transfer of contact sensitivity to picryl chloride, and their identity with the classical Ts-eff produced by injecting picrylsulphonic acid was suggested by their Thy-1.2 positivity and the liberation of TsF *in vitro*.

The production of TsF *in vitro* occurs when CBA mice are injected with picrylsulphonic acid and then re-exposed to antigen by painting with picryl chloride the day before harvesting. However painting with picryl chloride can be replaced by the injection of picrylated syngeneic $I\text{-}J^+$, but not $I\text{-}J^-$, cells or by picrylated cells from allogeneic mice (5R) which share the $I\text{-}J^k$ of CBA mice. A similar genetic restriction affected the liberation of non-specific inhibitor from armed T acceptor cells. Taken together these results show that syngeneic $I\text{-}J^+$ cells and allogeneic cells which share the I-J genotype of the recipient induce Ts-eff, cause Ts-eff to release TsF and trigger the Tacc armed with TsF to release non-specific inhibitor.

Why does the same I-J restriction occur at these three stages of the T suppressor circuit? The antigen specificity and the genetic restriction of the TsF suggests that this molecule has recognition sites for both conventional antigen (haptene) and for I-J (Zembala *et al.*, 1982b), and this is in keeping with the two-receptor model of associative recognition (Janeway, Wigzell & Binz, 1976). The original theory of antibody production proposed that the lymphocyte had antibody on its surface which it shed and resynthesized in increased amounts when exposed to antigen. It followed that the cellular receptor for antigen resembled the secreted product (Ehrlich, 1900). By analogy the receptor on the precursor of the Ts-eff and on the Ts-eff resembles TsF and hence has recognition sites for both I-J and for antigen. In other words, the genetic restriction conveyed by TsF in the triggering of the Tacc implies a similar restriction in the induction of the Ts-eff.

There are several studies of the induction of suppressor cells by the injection of cell-bound antigen. Miller, Sy & Claman (1978) showed that dinitrophenylated cells syngeneic with the recipient produced Ts-eff, while cells fully allogeneic at the major histocompatibility complex (MHC) only produced Ts-aff, i.e. cells which suppress when injected early in the immune response. Heavily picrylated (trinitrophenylated) cells produce Ts-aff which are genetically unrestricted in their induction and in the strain in which they act (Pierres *et al.*, 1980). However lightly picrylated cells are more effective in inducing Ts-aff when they are allogeneic with the recipient in the I-J region. This is probably due to an I-J allogeneic effect which favours the transition of primed Ts-aff into active suppressor cells (Bromberg, Benacerraf & Greene, 1981).

The suggestion that antigen on $I\text{-}J^+$ cells is an induction signal for suppressor cells arose from the finding that MHC antigens on cells with an allogeneic I-J genotype caused greater unresponsiveness than MHC antigens on cells with a syngeneic I- genotype. A summary of the evidence and its limitations is given by Mitchison (1981) and Liew (1981).

Streilein & Klein (1980) induced neonatal tolerance by injecting allogeneic cells and then testing by skin grafts of the same genotype. Unresponsiveness was more readily induced to the K and D loci when there was also a difference at the I-J locus, and differences in the Ia region usually induced suppressor cells (Streilein & Gruchalla, 1981). Liew (1981) found that Ts-aff, which depressed delayed hypersensitivity to K and D antigens, were only induced by cells of an allo-I-J genotype. Similarly Czitrom, Sunshine & Mitchison

(1980) found that I-J differences reduced the mixed lymphocyte reaction caused by differences at other loci. However Brondz, Karaulov, Chervonsky & Blandova (1982) demonstrated suppressor cells which depressed the mixed lymphocyte reaction and were induced by K and D in the absence of I-J differences. The ability of cells, differing only at K and D from the recipient, to induce T suppressor cells may be an example of the induction of suppressor cells by the presentation of antigen together with syngeneic I-J.

There is an apparent paradox. On the one hand, picrylated syngeneic I-J$^+$ cells are required to induce Ts-eff; on the other hand, allogeneic I-J is required to produce Ts in the transplantation antigen systems, with the exception of the studies of Brondz *et al.* (1982). We would like to suggest that the presentation of antigen on I-J$^+$ cells, whether syngeneic or allogeneic, is one of the induction signals for Ts-eff and certain other modes of unresponsiveness. In fact there are two issues: why is allogeneic I-J necessary in most transplantation systems, and why is allogeneic I-J ineffective in hapten systems? The work of Bromberg *et al.* (1981) shows that cells of an allogeneic I-J genotype favour the transition from primed Ts to active Ts. Perhaps this effect is critical for the induction of a detectable suppressor-cell response in certain transplantation systems.

The apparent failure of allogeneic cells to induce Ts-eff in the picryl system may be an example of pseudogenetic restriction. This concept, introduced by Okuda *et al.* (1981b), may be explained by reference to Fig. 1. This illustrates the view that the recognition (binding) sites of the TsF recognize the same I-J as that used to induce the Ts-eff. It follows that the TsF and Ts-eff can only be demonstrated when exposed to the same I-J as that used for their induction. However, in the present system all testing was undertaken using picrylated cells syngeneic with the Ts. It is possible that allo-I-J-directed Ts-eff may have been produced but not detected, and preliminary experiments show that this is indeed the case. In contrast, in the transplantation systems the allo-I-J genotype used to induce Ts was always present when the Ts was assayed. Further work will be necessary to prove whether these two concepts of the stimulatory effect of I-J differences on the development of T suppressor cells and of pseudogenetic restriction will allow the generalization that antigen on I-J, whether allogeneic or syngeneic, is the induction signal for all T suppressor circuits.

ACKNOWLEDGMENT

Vittorio Colizzi thanks the European Molecular Biology Organization for financial support.

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