SPECIFIC SUPPRESSION OF ALLOGRAFT REJECTION BY TRINITROPHENYL (TNP)-INDUCED SUPPRESSOR CELLS IN RECIPIENTS TREATED WITH TNP-HAPTENATED DONOR ALLOANTIGENS

By I. V. HUTCHINSON, W. H. BARBER, and P. J. MORRIS

From the Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DU, England

Suppressor T cells (Ts)[1] have been described in many allogeneic systems and may play a role in preventing allograft rejection in tolerized (1–5), antigen-pretreated (6–9), passively enhanced (10, 11), or cyclosporin A–treated (12, 13) recipients with long-term surviving transplants. The interactions between the Ts and their target cells are largely undefined. If Ts cells were specific for the appropriate alloantigenic receptor on antigen-reactive cells, i.e., antiidiotypic, they could react directly with alloreactive clones of cells capable of causing rejection. This form of suppression would be exquisitely specific as only clones of alloreactive cells bearing the appropriate receptors would be suppressed. Although strictly allospecific suppression has been described (9), our recent studies (14) show a distinct lack of specificity, particularly in active allograft enhancement models thought to be mediated by Ts. In these models, haplotype-specific suppression was able to protect a heterozygous transplant, and suppression of responses to major histocompatibility complex (MHC) antigens could be induced by pretreatment with minor alloantigens.

A simple model to explain the apparent lack of specificity is that Ts can effect their regulatory functions via an antigen bridge. Thus, if a Ts is specific for a "suppressor determinant" X on a multideterminant antigen expressing epitopes X, Y, and Z, the Ts will regulate the response to Y and Z antigens or any other determinant associated with the suppressor determinant, X. Therefore, the specificity of suppression observed in any given system depends on the antigen presented to the Ts and not solely on the precise specificity of the Ts itself. In this way, Ts in DA ($RT1^a$) rats with specificity for LEW ($RT1^l$) antigens will prevent rejection of (LEW × BN) F_1 kidneys but not of BN ($RT1^n$) allografts (14). Similarly, Ts specific for minor antigens can protect allografts across MHC barriers if the appropriate minor antigens are expressed on the graft (14).

This work was supported by grants from the Medical Research Council, UK and The National Kidney Research Fund. W. H. Barber is a University of Alabama Surgical Research Fellow. Address correspondence to I. V. Hutchinson.

[1] *Abbreviations used in this paper:* DNBS, dinitrobenzene sulphonic acid; DNP, dinitrophenyl; DTH, delayed-type hypersensitivity; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; MEM, minimal essential medium; MHC, major histocompatibility complex; RAR, rabbit anti-rat Ig antibody; TNBS, trinitrobenzene sulphonic acid; TNP, trinitrophenyl; Ts, suppressor T lymphocyte; TsF, suppressor T cell factor.

To extend this concept further, it should be possible to use an artificial suppressor determinant to manipulate allograft rejection. In this study we show that Ts specific for a hapten, trinitrophenyl (TNP), can suppress kidney graft rejection in recipients treated with TNP-donor alloantigens.

Materials and Methods

Animals. Rats of the DA ($RT1^a$), LEW ($RT1^l$), PVG/c ($RT1^c$) inbred strains were supplied by the Animal Unit of the John Radcliffe Hospital, Oxford. Female rats, 8–20 wk old, were used throughout.

Induction of Suppressor Cells. Suppressor cells were induced by treatment with the nascent, chemically reactive forms of two haptens. DA rats were injected intravenously with 20 mg of trinitrobenzene sulphonic acid (TNBS) (Sigma Fine Chemicals, London) or dinitrobenzene sulphonic acid (DNBS) (Eastman Kodak Co., Rochester, NY) dissolved in 1 ml saline, and adjusted to pH 8 using 1 M NaOH in saline.

Preparation of Haptenated Alloantigens. Spleen cells suspensions from LEW or PVG rats were freed of erythrocytes using Gey's hemolytic buffer. The leukocytes were then incubated at 10^8 cells/ml in 1 mM TNBS, 1 mM DNBS, or 100 μg/ml fluorescein isothiocyanate (FITC) (Sigma Fine Chemicals) in phosphate-buffered saline for 10 min at 37°C. The cells were washed three times using minimal essential medium (MEM) with 5% fetal calf serum (FCS), resuspended at 10^8 cells/ml in MEM-FCS, ultrasonicated at 20 kHz on ice (4 × 20 s at 100 W), and centrifuged at 2,000 *g* for 10 min. The supernatant fluid containing the haptenated alloantigenic preparation was stored in small aliquots at −20°C.

Adoptive Transfer of Cells. Hapten-activated suppressor cells were assayed by adoptive transfer to sublethally irradiated (100, 200, or 400 rad x ray delivered at 133 rad/min through a 1.4 mm copper filter) syngeneic DA rats. Spleen, thymus, and lymph node cell suspensions were prepared 2 d to 12 wk after injection of nascent haptens. The cells were washed in MEM-FCS but, except where stated, were otherwise untreated. Usually, 10^8 cells (or the number of cells recovered from a separation procedure starting with 10^8 cells) were transferred into each recipient rat.

Separation of Cells. The cell surface phenotype of the suppressor cells was studied using several separation techniques. Immunoglobulin-positive (Ig^+) B cells were depleted by panning on plates coated with affinity-purified rabbit anti-rat (RAR) Ig antibody (15). The depleted cells were >98% Ig^- when examined by FITC-RAR labeling and flow cytometry. In some groups the panned Ig^- cells were further fractionated by incubation with either W3/25 (T helper cell) or OX8 (T cytotoxic/suppressor cell) antibody and rosetting with sheep erythrocytes coated with anti-rat Ig (16). Nylon wool–adherent cells were removed according to the murine method (15). Nylon wool–nonadherent cells were ~98% Ig^- by FITC-RAR Ig labeling and ultraviolet light microscopy. However, there was a considerable loss of cells during this procedure. Routinely, 23–36% of the cells applied to the nylon wool column elute in the nonadherent fraction, compared with the 46–52% of cells in the DA spleen that are Ig^- or Ia^- (Hutchinson, unpublished results). Hence, one-third to one-half of the Ig^- cells were lost at this stage. In another experiment, putative suppressor cells were incubated at 20°C for 30 min in a 1:100 dilution of W3/13 pan T cell monoclonal ascites exudate antibody (Sera Labs, Crawley, UK) at 10^8 cells/ml, followed by the addition of an equal volume of rabbit complement diluted 1:5 and incubation for 60 min at 37°C.

Drugs and Irradiation of Cells. Cyclophosphamide (Farmitalia Carlo Erba Ltd., Barnet, England) was given intraperitoneally at a dose of 50 mg/kg; cortisone (Cortistab; The Boots Company plc, Nottingham, England) was given intraperitoneally at 100 mg/kg. Cells were irradiated with 1,000 rad gamma rays from a cesium 137 source (Gamma Cell 1000; Atomic Energy of Canada, Ltd., Ottawa) at a rate of 450 rad/min.

Kidney Transplantation. The left kidney of DA recipients was removed and replaced with a kidney from LEW or PVG donors, using end-to-end anastomoses of artery, vein, and ureter. The operation was performed under ether/chloral anesthesia and warm

ischemia was generally 20–30 min. 1 wk after transplantation, the recipient's own remaining (right) kidney was removed so that, from then on, survival of the rat was dependent on graft function. This delayed contralateral nephrectomy model prevents the occasional long-term survival of untreated recipients due to uremia-induced immunosuppression in the first week, and enables inspection of the transplant on day 7.

Experimental Protocol. Recipient DA rats were irradiated 2 d before transplant and received 10^8 suppressor cells and hapten-modified alloantigen derived from 10^7 spleen cells the day before transplantation.

Results

Suppression of Allograft Rejection by TNBS-induced Suppressor Cells. Adoptive transfer of normal DA spleen cells to 400-rad–irradiated syngeneic rats either with or without TNP-LEW antigen (Table I, groups S2 and S1, respectively) did not cause long-term survival of subsequent LEW renal allografts. However, when spleen cells from TNBS-pretreated rats were transferred with TNP-LEW antigen, the majority of recipients survived for >100 d (Table I, groups S4–S8). Pooling all data (group S4), it can be seen that, of 43 rats, 34 (79%) survived >100 d and 37 (86%) for >50 d.

Appearance of Suppressor Cells in Spleen, Thymus, and Lymph Nodes after TNBS Treatment. The time course of the appearance of suppressor cells differed from one lymphoid compartment to another. There were effective suppressor cells in the spleen within 2 d of TNBS injection (Table I, group S5) and they were still present 12 wk after activation. We have routinely transferred splenic suppressor cells from rats pretreated with TNBS 7–21 d previously (Table I, groups S6–S8 and Tables II–V). By contrast, the most effective suppression with thymus or lymph nodes cells was observed at 2 wk postinduction (Table I, groups T5 and LN5), and this was particularly evident in the case of lymph node cells.

Radiation Dependence of Adoptive Transfer of Suppression. There was a requirement for sublethal irradiation for the adoptive cell transfer. Rats routinely received 400 rad but 200 would suffice (group S10), while rats given either 100 rad (group S12) or no irradiation (group S14) did not accept the subsequent allografts.

Cell Surface Phenotype of the Splenic Suppressor Cells. Spleen cells from DA rats pretreated with TNBS 7–21 d previously were fractionated and tested for suppressor cell activity. Removal of Ig^+ cells by panning on anti-Ig plates did not deplete suppressive activity (Table II, group 5) whereas removal of T cells with the pan T cell antibody W3/13 and complement did (group 8). Fractionation by rosetting to remove $W3/25^+$ cells failed to abrogate suppressive activity (group 6). We conclude that the cell responsible for suppression is an $OX8^+$ T cell, although, strictly, it is a Ig^-, $W3/25^-$ cell. The suppressor cell was adherent to nylon wool (group 9), a charateristic that has been reported for populations of activated Ts cells in the mouse (18, 19).

Drug and Radiation Sensitivity of TNBS-activated Suppressor Cells. Spleen cells from rats given TNBS 14 d before transfer and 50 mg/kg cyclophosphamide 2 d before transfer were not suppressive (Table III, group 3) indicating their sensitivity to this drug. Similarly, irradiation with 1,000 rad gamma rays immediately before transfer abrogated the suppressor activity of either spleen cells or thymus cells (both 7 d after TNBS) (Table III, groups 5 and 9, respectively).

TABLE I

Suppression of LEW to DA Kidney Allograft Rejection by Spleen, Thymus, and Lymph Node Cells from Normal or TNBS-treated DA Rats

Group	x Radiation*	Cells transferred‡	Antigen§	n‖	Survival¶	MST**	P‡‡
	rad				*d*		
C1	400	None	None	5	10, 10, 11, 11, 11	11	
C2	400	None	TNP-LEW	5	11, 12, 12, 14, 22	12	
S1	400	Normal spleen	None	5	10, 10, 11, 11, 16	11	
S2	400	Normal spleen	TNP-LEW	5	11, 16, 17, 17, 18	17	
S3	400	TNBS spleen (7–21)	None	5	10, 10, 10, 12, 14	10	
S4	400	TNBS spleen (2–84)	TNP-LEW	43	14, 16, 17, 19, 27, 35, 35, 55, 63, >100 (×34)	>100	<0.001
S5	400	TNBS spleen (2)	TNP-LEW	5	27, >100 (×4)	>100	<0.001
S6	400	TNBS spleen (9)	TNP-LEW	5	17, >100 (×4)	>100	<0.02
S7	400	TNBS spleen (14–16)	TNP-LEW	7	19, 55, 63, >100 (×4)	>100	<0.001
S8	400	TNBS spleen (19–21)	TNP-LEW	4	>100 (×4)	>100	<0.001
S9	200	Normal spleen	TNP-LEW	5	10, 11, 12, 12, 13	12	
S10	200	TNBS spleen (7–21)	TNP-LEW	6	15, >100 (×5)	>100	<0.001
S11	100	Normal spleen	TNP-LEW	6	10, 10, 10, 11, 11, 13	10	
S12	100	TNBS spleen (7–21)	TNP-LEW	4	10, 11, 14, >100	12	
S13	None	Normal spleen	TNP-LEW	5	10, 10, 10, 10, 11	10	
S14	None	TNBS spleen (7–21)	TNP-LEW	5	10, 10, 10, 11, 11	10	
T1	400	Normal thymus	None	5	10, 11, 12, 15, 21	12	
T2	400	Normal thymus	TNP-LEW	7	10, 11, 12, 14, 14, 16, 20	14	
T3	400	TNBS thymus (9)	None	4	11, 13, 14, 18	13	
T4	400	TNBS thymus (6–7)	TNP-LEW	5	19, 32, 42, 50, >100	42	<0.005
T5	400	TNBS thymus (14–16)	TNP-LEW	6	14, 41, >100 (×4)	>100	<0.005
T6	400	TNBS thymus (19–22)	TNP-LEW	6	20, 21, 23, 25, >100 (×2)	24	<0.005
LN1	400	Normal LN	None	5	14, 14, 15, 16, 18	15	
LN2	400	Normal LN	TNP-LEW	5	12, 13, 14, 15, 22	14	—
LN3	400	TNBS LN (9)	None	4	12, 13, 16, 17	14	
LN4	400	TNBS LN (7–9)	TNP-LEW	3	10, 14, 16	14	
LN5	400	TNBS LN (14–16)	TNP-LEW	7	12, 18, >100 (×5)	>100	<0.05

TABLE I (*continued*)

Group	x Radiation*	Cells transferred‡	Antigen§	n‖	Survival¶	MST**	P‡‡
	rad				*d*		
LN6	400	TNBS LN (19–22)	TNP-LEW	7	10, 11, 11, 13, >100 (×3)	13	

* DA rats were sublethally irradiated with 100, 200, or 400 rad x rays (except groups S13 and 14) 1 d before adoptive cell transfer.

‡ Cell suspensions were prepared from the spleens, thymuses, or lymph nodes of normal or TNBS-pretreated DA rats. 10^8 cells were transferred to each recipient. The number in parenthesis indicates how many days previously the cell donors had been pretreated with TNBS.

§ Antigen, prepared from 10^7 TNP-haptenated LEW spleen leukocytes by sonication, were injected intravenously at the same time as the cell inoculum.

‖ Number in group.

¶ The day after adoptive cell transfer each rat received a kidney allograft from LEW strain donors. The survival of individuals (in days) after transplant is given.

** Median survival time.

‡‡ Statistical significance of difference between groups S2 and S3–14, group T2 and groups T3–6, and group LN2 and groups LN3–6, using the Mann-Whitney U test.

TABLE II

Cell Surface Phenotype of TNBS-activated Spleen Cells that Suppress Rejection of LEW Kidney Grafts by DA Rats Treated with TNP-Donor Antigen

Group	Cells transferred*	Antigen‡	n‡	Survival (days)‡	MST‡	P§
1	Normal unfractionated	TNP-LEW	5	11, 16, 17, 17, 18	17	—
2	Normal panned T cells	TNP-LEW	5	11, 17, 19, 20, 35	19	NS
3	Normal $OX8^+$ T cells	None	5	11, 12, 12, 14, 18	12	NS
4	TNBS unfractionated	TNP-LEW	16	17, 19, 55, 63, >100 (×12)	>100	<0.001
5	TNBS Ig^- T cells	TNP-LEW	5	>100 (×5)	>100	<0.001
6	TNBS $W3/25^-$ T cells (OX8)	TNP-LEW	5	33, >100 (×4)	>100	<0.001
7	TNBS + C	TNP-LEW	6	>100 (×6)	>100	<0.001
8	TNBS + W3/13 + C	TNP-LEW	5	10, 14, 19, 20, 23	19	NS
9	TNBS nylon wool nonadherent	TNP-LEW	4	14, 17, 25, >50	21	NS

* Spleen cells from normal or TNBS-treated DA rats were fractionated before adoptive transfer to 400-rad–irradiated syngeneic recipients. Fractionation procedures were: panning on anti-Ig plates, rosetting to remove $W3/25^+$ cells, treatment with rabbit complement or with W3/13 antibody and complement, or nylon wool adherence (see text).

‡ See footnotes to Table I.

§ Statistical significance compared with group 1 using the Mann-Whitney U test. NS, not significant.

Finally, the Ts cells were shown to be cortisone resistant. Thymus cells from rats given TNBS 2 wk before transfer and 100 mg/kg cortisone 2 d before transfer were still able to prolong renal allograft survival (Table III, group 8).

Allospecificity of Suppression Mediated by TNBS-activated Suppressor Cells. According to our hypothesis that suppression in this system is mediated by an antigen bridge, the specificity of suppression will depend on the epitopes present

TABLE III

Drug and Radiation Sensitivity of TNBS-activated Spleen Suppressor Cells Transferred to DA Recipients Using LEW Kidneys

Group	Cells transferred*	Antigen‡	n‡	Survival (days)‡	MST‡	P§
1	Normal spleen	TNP-LEW	5	11, 16, 17, 17, 18	17	
2	TNBS spleen	TNP-LEW	7	19, 55, 63, >100 (×4)	>100	<0.001
3	Cy TNBS spleen	TNP-LEW	4	11, 15, 16, 17	15	NS
4	TNBS spleen	TNP-LEW	5	17, >100 (×4)	>100	0.016
5	1,000 rad TNBS spleen	TNP-LEW	7	12, 12, 14, 15, 17, 19	14	NS
6	Normal thymus	TNP-LEW	7	10, 11, 12, 14, 14, 16, 20	14	NS
7	TNBS thymus	TNP-LEW	5	19, 32, 42, >100 (×2)	42	0.003
8	HC TNBS thymus	TNP-LEW	4	24, >100 (×3)	>100	<0.001
9	1,000 rad TNBS thymus	TNP-LEW	5	10, 13, 13, 20, 27	13	NS

* Spleen or thymus cells from DA rats treated with TNBS 14 d previously were adoptively transferred after either drug treatment of the cell donors or irradiation of the cells immediately before transfer. In group 3, cell donors received 50 mg/kg cyclophosphamide (Cy) 2 d before spleen cell transfer. In group 8, cell donors were given 100 mg/kg hydrocortisone (HC) 2 d before thymus cell transfer. In groups 5 and 8, cells were irradiated with 1,000 rad gamma rays before transfer.

‡ See footnotes to Table I.

§ Statistical significance comparing groups 2–5 with group 1, and 7–9 with group 6, using the Mann-Whitney U test. NS, not significant.

TABLE IV

Allospecificity of Suppression Mediated by TNBS-activated Splenic Suppressor Cells Transferred to DA Recipients before Receiving a LEW or PVG Kidney

Group	Cells*	Antigen‡	Kidney§	n‖	Survival (days)¶	MST**	P‡‡
1	TNBS	TNP-LEW	LEW	16	17, 19, >50 (×14)	>50	<0.001
2	TNBS	TNP-PVG	LEW	5	11, 15, 17, 20, 28	17	
3	TNBS	TNP-LEW	PVG	5	11, 15, 17, 17, 20	20	<0.005
4	TNBS	TNP-PVG	PVG	8	16, 22, 46, >50 (×5)	>50	

* Spleen cells from DA rats pretreated with TNBS 7–21 d beforehand were adoptively transferred into 400-rad–irradiated syngeneic recipients.

‡ TNP-haptenated antigens, prepared from either LEW or PVG spleen leukocytes, were injected with the cell inoculum.

§ The day after adoptive cell transfer, recipient rats were given a LEW or PVG renal allograft.

‖ Number in group.

¶ Individual survival times in days.

** Median survival time.

‡‡ Statistical significance of the comparisons between groups 1 and 2 or 3 and 4 using the Mann-Whitney U test.

on the bridging antigen. Hence TNP-specific cells will protect LEW kidneys in rats treated with TNP-LEW but not TNP-PVG (third-party) alloantigen, whereas rats given TNBS-activated spleen cells and TNP-PVG antigen rejected LEW kidneys (Table IV, group 2). In the reciprocal groups, rats given TNP-induced suppressor cells and TNP-PVG antigen retained a PVG kidney (Table IV, group 4) but those given TNP-LEW antigen were responsive to PVG antigens, rejecting the PVG kidney (group 3).

Haptenic Specificity of TNBS-induced Suppressor Cells. The argument that the specificity of suppression will depend on epitopes on the antigen bridge applies

TABLE V

Haptenic Specificity of Suppression Mediated by Hapten-activated Suppressor Cells Transferred to DA Recipients Treated with Hapten-conjugated Donor Alloantigen before Receiving LEW Kidneys.

Group	Cells*	Antigen‡	n§	Survival (days)§	MST§	P‖
1	None	None	5	10, 10, 11, 11, 11	11	
2	None	DNP-LEW	5	10, 13, 15, 19, 21	15	
3	None	TNP-LEW	5	11, 12, 12, 14, 22	12	
4	None	FITC-LEW	3	12, 14, 18	14	
5	Normal	None	5	10, 10, 11, 11, 16	11	NS
6	Normal	DNP-LEW	5	12, 13, 14, 18, 20	14	NS
7	Normal	TNP-LEW	5	11, 16, 17, 17, 18	17	NS
8	Normal	FITC-LEW	4	11, 13, 17, 17	15	NS
9	DNBS	None	6	11, 13, 14, 14, 17, 17	14	NS
10	DNBS	DNP-LEW	7	14, 27, >100 (×5)	>100	<0.02
11	DNBS	TNP-LEW	7	10, 14, 15, 17, 21, >100 (×2)	17	NS
12	TNBS	None	5	10, 10, 10, 12, 14	10	NS
13	TNBS	DNP-LEW	7	11, >100 (×6)	>100	<0.02
14	TNBS	TNP-LEW	16	17, 19, 55, 63, >100 (×12)	>100	<0.001
15	TNBS	FITC-LEW	5	10, 11, 13, 14, 18	13	NS
16	TNBS	LEW	5	10, 11, 14, 22, >50	14	NS
17	TNBS	TNP-PVG+LEW	5	10, 19, 45, >100 (×2)	45	NS

* Spleen cells from DA rats treated with DNBS or TNBS (see text).

‡ LEW spleen cell antigen, either unmodified (group 16) or haptenated with DNP, TNP, or fluorescein (FITC), was injected intravenously at the time of adoptive cell transfer.

§ See footnotes to Table I.

‖ Statistical significance of difference between groups 1 and 5, 9, and 12; groups 2 and 6, 10, and 13; groups 3 and 7, 11, 14, and 17; and groups 4 and 8 and 15. NS, not significant.

equally to the suppressor cell and the target of suppression. Thus, TNP-specific suppressors should suppress alloreactive cells only in the presence of TNP-haptenated alloantigens.

We have tested dinitrophenyl (DNP)- and TNP-activated suppressor cells transferred with DNP-, TNP-, or FITC-haptenated alloantigens in this kidney allograft model. TNBS-activated cells suppressed graft rejection in rats treated with DNP- or TNP-modified alloantigens (Table V, groups 13 and 14) but not in rats given FITC-haptenated or unmodified antigen (Table V, groups 15 and 16). DNBS-activated Ts suppressed rejection of grafts in rats given DNP-haptenated alloantigen (group 10) but there was only a weak crossreaction with TNP-haptenated donor antigen in this direction, and five of seven rats given DNP-Ts and TNP-alloantigen rejected their grafts (group 11). We have found that, for effective suppression, the hapten and the alloantigen have to be covalently linked. A mixture of unmodified LEW antigen and TNP-haptenated PVG antigen induced long-term survival of only two of five LEW kidneys (Table V, group 17).

Discussion

The ability of helper T cells specific for one epitope on a multi-determinant antigen to help the response to another epitope has long been accepted. The classic example is the hapten-carrier effect in B cell activation (20). It is clear that similar interactions occur in the activation of T cells and that the epitopes need not be on the same molecule. For instance, in the in vitro mixed lymphocyte reaction, class II MHC antigen-specific T helper cells can play a role in the activation of cytotoxic T lymphocytes specific for class I MHC antigens on the surface of stimulator cells expressing both class I and II products (21). Suppression, too, can be mediated via an antigen bridge so that suppressor cells specific for one epitope can suppress responses to another. This has been shown for both B and T cell responses to hen egg lysozyme (22) and other antigens (23, 24), and may represent an important component in immune regulation.

We wished to examine suppression of T cell responses via antigen bridges in allogeneic systems. Suppression of delayed-type hypersensitivity (DTH) to MHC antigens (25) and allograft rejection (14) can be brought about by pretreatment with minor alloantigens, an effect probably mediated by Ts specific for these minor allodeterminants. The complexity of such systems makes detailed analyses difficult, especially as minor transplantation antigen systems have not been extensively characterized in species other than the mouse.

Studies in mice have shown that DTH responses to the hapten TNP can be suppressed by T cells from TNP-tolerant animals (26). TNP is a small, chemically defined molecule that can be covalently coupled with ease to transplantation antigens without destroying alloantigenic activity (27). The biochemistry of this reaction is well documented and TNP-coupled antigens have been used in studies of cytotoxic T lymphocyte activation (28, 29). In addition, the suppression mediated by TNP-activated Ts in DTH models has been extensively studied in mice (30, 31). We have shown that suppression of hapten-specific DTH in the rat is more pronounced than it is in the mouse.[2] Thus we chose to use a model system in which TNP-specific suppressor cells were used, in combined treatment with TNP-alloantigen, to suppress rat renal allograft rejection.

Spleen cells from TNBS-treated DA rats do protect LEW kidneys in recipients treated with TNP-LEW antigen (Table I, group S4). They appear in the spleen within 2 d of TNBS injection and persist for up to 12 wk. However, they are sensitive to both cyclophosphamide and radiation (Table III, groups 3 and 5), which may indicate that they have to divide in response to TNP-alloantigen in the adoptive cell transfer recipient to exert their suppressive influence.

Suppressor cells are to be found in the thymus and lymph nodes after TNBS injection but their appearance is relatively transient, especially in lymph nodes, when compared with the spleen (Table I, groups T4–T6 and LN4–6). The thymocytes are a mature subpopulation, in being cortisone resistant (Table III, group 8), and their function, like that of splenocytes, is radiation sensitive (Table III, group 9). The distribution and kinetics of appearance of these suppressors indicates that they belong to the mature, sessile, spleen-seeking T1 category of T cells (32), and are activated in situ. The transient appearance in lymph nodes

[2] Prop, J., A. G. Griffiths, I. V. Hutchinson, and P. J. Morris. Specific suppressor T cells in rats active in the afferent phase of contact sensitivity. Submitted for publication.

may, however, reflect a different balance of help and suppression compared with the spleen rather than a quirk in recirculation potential. We have not examined the phenotype of lymph node suppressor populations.

The cell surface phenotype of spleen cell populations was examined in the period 7–21 d after TNBS injection, and it would appear that the suppression is mediated by T cells. Removal of Ig^+ cells by panning did not adversely affect suppressor function (Table II, group 5), whereas removal of T cells did (Table II, group 8). Nor did removal of $W3/25^+$ cells abrogate suppression (Table II, group 6). We have evidence from other systems that the initial activation of Ts requires help (14). The observation that W3/25-depleted T cells (presumably therefore $OX8^+$ cells) suppress in this system suggests that they do not need TNP-primed help after adoptive transfer, although $W3/25^+$ cells in the recipient, perhaps activated by subsequent allografting, may be necessary. The T cell nature of the suppressor cells is supported by the finding that they carry the W3/13 pan T cell marker. Parenthetically, the W3/13 monoclonal antibody is of the mouse IgG1 subclass, which poorly fixes complement. However, in high concentration and with rabbit complement (which contains heterophile antibodies), a subpopulation of $W3/13^+$ cells is lysed. The interpretation of this finding is complicated by the reported presence of the W3/13 antigen on some plasma cells (33).

The adherence of the suppressor cells to nylon wool is in accord with previous reports of activated Ts cells in the mouse (18, 19). The nylon wool method was originally developed for murine cell fractionation (17). When used with rat cells under conditions that deplete B cells, there is also a considerable loss of T cells (see Materials and Methods). Normal nonadherent splenocytes respond well in mixed leukocyte response assays (Hutchinson, unpublished results) and Ig^- suppressor cells from long-surviving, passively enhanced renal allograft recipients are not nylon wool adherent (11). By contrast, these TNP-activated suppressor cells are like the Ts cells described in some other allogeneic (18, 34) and hapten-specific (19) systems, in that they appear to be nylon wool adherent.

Our conclusion, then, is that the suppressor cells in this system are idiotype (TNP)-specific, activated $OX8^+$ T cells which need to divide to exert their effects and which proliferate in the absence of TNP-primed $W3/25^+$ helper T cells.

The mechanism whereby TNP-specific Ts can suppress allograft rejection remains a matter for speculation. The "antigen bridge" concept is an obvious oversimplification and we have begun studies into the details of the suppressive cell interactions. The first question is whether these cells produce soluble factors, and our preliminary studies suggest that they do. Supernatants from overnight cultures of TNBS-activated spleen cells restimulated with TNP-haptenated syngeneic cells are suppressive in vivo preventing rejection of LEW kidneys in DA rats treated with TNP-LEW alloantigen. Although we have not yet tested the factor alone, it is unlikely to act nonspecifically because LEW kidneys are not protected by factor plus TNP-PVG (third party) alloantigen. Other preliminary results suggest that the factor is a glycoprotein that may bind antigen. Suppressive activity is found in fractions of molecular weight $<100,000$, in agreement with our conclusion that suppression is mediated by T cells rather than antibodies. Our working hypothesis is that this suppression acts at the level of the antigen-

presenting cell, preventing activation of T helper cells necessary for the rejection response. This idea fits with our findings in a rat DTH model that these cells act in the afferent rather than efferent phase.[2]

In other systems, Ts cells can be placed in various steps of a rather complex pathway (35, 36). It is proposed that a factor (TsF1) produced by an idiotype-positive cell (Ts1) activates an antiidiotypic (Ts2) cell which, in turn, elaborates a factor (TsF2). This factor activates idiotype-positive Ts3 cells to produce a nonspecifically acting TsF3. The TNP-activated Ts described in this paper fit the Ts1 category. However, our current hypothesis is that Ts3 cells are simply differentiated Ts1 cells, a process blocked by irradiation or cyclophosphamide and augmented by antiidiotypic Ts2 cells. We have some evidence derived from allogeneic systems to support this view, which will be presented separately.

Finally, the question arises as to how the transient influence of TNP-Ts and TNP-donor alloantigen can cause indefinite survival of allografts. Adoptive transfer assays show that these long-term survivors have allospecific Ts in their spleens and residing in their transplants.[3] These are activated by the transplant; perhaps any treatment that temporarily interferes with helper cell activation, including active or passive enhancement and treatment with donor-specific complexes or with cyclosporine, will allow ascendency of the suppressor cell population.

Summary

Suppressor T cells, activated by injection of trinitrobenzene sulphonic acid in DA rats, prevented rejection of LEW kidney allografts in a donor-specific manner when adoptively transferred into syngeneic recipients along with trinitrophenyl (TNP)-haptenated LEW alloantigen. TNP-haptenated third-party alloantigen was ineffective in this system. The donor-specific suppression was dependent, too, on the haptenic portion of the chemically modified alloantigen. Hence, fluorescein isothiocyanate–donor antigen did not lead to suppression in the presence of TNP-reactive suppressor cells. There is, however, some crossreaction between DNP- and TNP-haptenated alloantigens so that TNP-reactive cells and DNP–donor antigen suppressed rejection whereas DNP-reactive cells and TNP–donor antigen did not prevent graft rejection. The suppressor cells were sensitive to cyclophosphamide and radiation but were resistant to hydrocortisone. They appear to be T cells of the OX8 (suppressor/cytotoxic) phenotype since they are positive for the pan T cell antigen W3/13, are Ig negative, and do not carry the W3/25 (T helper cell) marker. However, these suppressor cells are adherent to nylon wool. They are found mainly in the spleen, are detected there within 2 d of TNBS injection, and can persist for up to 12 wk. We propose that these cells are first-order T suppressor (Ts1) cells that act in the afferent phase of the response to a renal allograft.

Received for publication 1 July 1985.

[3] Hutchinson, I. V., W. H. Barber, and P. J. Morris. Mechanisms maintaining long-term survival of rat kidney allografts. Submitted for publication.

References

1. Dorsch, S., and B. Roser. 1975. T cells mediate transplantation tolerance. *Nature (Lond.)*. 258:233.
2. Dorsch, S., and B. Roser. 1977. Recirculating suppressor T cells in transplantation tolerance. *J. Exp. Med.* 145:1144.
3. Gruchalla, R. S., and J. W. Streilein. 1981. Transplantation tolerance: clonal deletion, active suppression, or both? *Transplant. Proc.* 13:574.
4. Tutschka, P. J., R. Belanger, A. D. Hess, W. R. Beschorner, and G. W. Santos. 1983. Suppressor cells in transplantation tolerance to self and non-self. *Transplant. Proc.* 15:853.
5. Stephenson, P., S. Dorsch, B. Roser, U. Godden, and J. Herbert. 1983. The identity of suppressor cells in neonatal tolerance. *Transplant. Proc.* 15:850.
6. Stuart, F. P., D. M. Scollard, T. J. McKearn, and F. W. Fitch. 1976. Cellular and humoral immunity after allogeneic renal transplantation in the rat. *Transplantation (Baltimore)*. 22:455.
7. Hendry, W. S., N. L. Tilney, W. M. Baldwin, M. J. Graves, E. Milford, T. B. Strom, and C. B. Carpenter. 1979. Transfer of specific unresponsiveness to organ allografts by thymocytes: specific unresponsiveness by thymocyte transfer. *J. Exp. Med.* 149:1042.
8. Horsburgh, T., P. J. Wood, and L. Brent. 1981. In vivo induction of specific suppressor lymphocytes to histocompatibility antigens. *Transplant. Proc.* 13:637.
9. Marquet, R. L., and G. A. Heystek. 1981. Induction of suppressor cells by donor-specific blood transfusions and heart transplantation in the rat. *Transplantation (Baltimore)*. 31:272.
10. Batchelor, J. R., B. E. Phillips, and D. Grennan. 1984. Suppressor cells and their role in the survival of immunologically enhanced rat kidney allografts. *Transplantation (Baltimore)*. 37:43.
11. Barber, W. H., I. V. Hutchinson, and P. J. Morris. 1984. The role of suppressor cells in maintaining passively enhanced rat kidney allografts. *Transplantation (Baltimore)*. 38:548.
12. Bordes-Aznar, J., J. W. Kupiec-Weglinski, A. J. S. Duarte, E. L. Milford, T. B. Strom, and N. L. Tilney. 1983. Function and migration of suppressor lymphocytes from cyclosporine-treated heart graft recipients. *Transplantation (Baltimore)*. 35:185.
13. Barber, W. H., I. V. Hutchinson, and P. J. Morris. 1985. Mechanisms of kidney allograft maintenance in rats treated with cyclosporine. *Transplant. Proc.* 17:1391.
14. Hutchinson, I. V., and P. J. Morris. The role of major and minor histocompatibility antigens in active enhancement of rat kidney allograft survival by blood transfusion. *Transplantation (Baltimore)*. In press.
15. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation on polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods.* 15:47.
16. Mason, D. W. 1981. Subsets of T cells in the rat mediating lethal graft-versus-host disease. *Transplantation (Baltimore)* 32:222.
17. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
18. Holan, V., J. Chutna, and M. Hasek. 1978. Specific suppression of antigen reactive cells in neonatal transplantation tolerance. *Nature (Lond.)*. 274:895.
19. Tada, T., M. Taniguchi, and T. Takemori. 1975. Properties of primed suppressor T cells and their products. *Transplant. Rev.* 26:106.

20. Mitchison, N. A. 1971. The carrier effect in the secondary response to hapten-carrier conjugates. *Eur. J. Immunol.* 1:10.
21. Bach, F. H., M. Bach, and P. M. Sondel. 1976. Differential function of major histocompatibility complex antigens in T-lymphocyte activation. *Nature (Lond.).* 259:273.
22. Sercarz, E. E., R. L. Yowell, D. Turkin, A. Miller, B. A. Araneo, and L. Adorini. 1978. Different functional specificity repertoires for suppressor and helper T cells. *Immunol. Rev.* 39:108.
23. Debre, P., J. A. Kapp, and B. Benacerraf. 1975. Genetic control of specific immune suppression. I. Experimental conditions for the stimulation of suppressor cells by the copolymer L-glutamic acid^{50}-L-tyrosine^{50} (GT) in non-responder BALB/c mice. *J. Exp. Med.* 142:1436.
24. Herzenberg, L. A., T. Tokuhisa, K. Hayakawa, and L. A. Herzenberg. 1982. Lack of immune response gene control for induction of epitope-specific suppression by TGAL antigen. *Nature (Lond.).* 295:329.
25. Bianchi, A. T. J., L. M. Hussaarts-Odijk, and T. Benner. 1983. Antigen-specific suppressor T cells suppress in vivo the cellular immune response to unrelated "bystander" antigens. *Transplant. Proc.* 15:76.
26. Asherson, G. L., M. Zembala, W. R. Thomas, and M. A. C. C. Perera. 1980. Suppressor cells and the handling of antigen. *Immunol. Rev.* 50:3.
27. Forman, J., E. S. Vitetta, and D. A. Hart. 1977. Relationship between trinitrophenyl and H-2 antigens on trinitrophenyl-modified spleen cells. I. H-2 antigens on cells treated with trinitrobenzene sulfonic acid are derivatized. *J. Immunol.* 118:797.
28. Forman, J. 1975. On the role of the H-2 histocompatibility complex in determining the specificity of cytotoxic effector cells sensitized against trinitrophenyl-modified targets. *J. Exp. Med.* 142:403.
29. Shearer, G. M., T. G. Rehn, and C. Garbarine. 1975. Cell-mediated lympholysis of trinitrophenyl-modified autologous lymphocytes. Effector cell specificity to modified cell surface components controlled by the H-2K and H-2D serological regions of the murine major histocompatibility complex. *J. Exp. Med.* 141:1348.
30. Miller, S. D., M.-S. Sy, and H. N. Claman. 1978. Suppressor T cell mechanisms in contact sensitivity. I. Efferent blockade by syninduced suppressor T cells. *J. Immunol.* 121:265.
31. Claman, H. N., S. D. Miller, M.-S Sy, and J. W. Moorhead. 1980. Suppressive mechanisms involving sensitization and tolerance in contact allergy. *Immunol. Rev.* 50:105.
32. Raff, M. C., and H. Cantor. 1971. Subpopulations of thymus cells and thymus-derived lymphocytes. *In* Progress in Immunology. O. B. Amos, editor. Academic Press, Inc., New York.
33. Hess, A. D., P. J. Tutschka, and G. W. Santos. 1981. Effect of cyclosporin A on human lymphocyte responses in vitro. II. Induction of specific alloantigen unresponsiveness mediated by a nylon wool adherent suppressor cell. *J. Immunol.* 126:961.
34. Dyer, M. J. S., and S. V. Hunt. 1981. Committed T lymphocyte stem cells of rats. Characterization by surface W3/13 antigen and radiosensitivity. *J. Exp. Med.* 154:1164.
35. Germain, R. N., and B. Benacerraf. 1981. A single major pathway of T-lymphocyte interactions in antigen-specific immune suppression. *Scand. J. Immunol.* 13:1.
36. Dorf, M. E., and B. Benacerraf. 1984. Suppressor cells and immunoregulation. *Annu. Rev. Immunol.* 2:127.