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Regulatory role of suppressor T cells in the expression of delayed-type hypersensitivity in mice

I. TRANSIENT APPEARANCE OF SUPPRESSOR T CELLS FOR THE EXPRESSION OF DELAYED FOOTPAD REACTION INDUCED WITH LIPID-CONJUGATED LYSOZYME*

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Summary. Delayed footpad reaction (FPR) to lysozyme (Lys) in mice was induced without antibody responses by lipid-conjugated lysozyme (D.Lys). This FPR was suppressed by priming s.c. with a high dose (10 mg) of Lys 2 weeks previously (unresponsiveness). Spleen cells from the unresponsive mice suppressed antigen-specifically FPR in mice previously immunized with D.Lys, and also suppressed passive transfer of FPR by D.Lys-immune lymphoid cells into normal mice. The suppressive activity of the spleen cells was abolished by treatment with anti-θ antiserum and complement. The suppressor cells occurred also in the thymus of unresponsive mice. Unresponsiveness was induced in mice immediately after priming with Lys and persisted at least up to 7 weeks after the induction. In contrast, suppressor cells appeared only 2 weeks after induction of unresponsiveness in both the spleen and the thymus but were no longer detectable 3–7 weeks later, although donor mice remained fully unresponsive. These results suggest that antigen-specific suppressor T cells are involved in the regulation of the expression of FPR only for a definite period of time in unresponsive mice.

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INTRODUCTION

In recent years increasing attention has been paid to suppressor T cells which provide an important regulatory mechanism in many immune responses. Suppressor T cells involved in contact sensitivity to picryl chloride (Zembala & Asherson, 1973) and to dinitrofluorobenzene (DNFB) (Phanuphak, Moorhead & Claman, 1974) have been described in mice. Delayed footpad reaction (FPR), which is another manifestation of delayed-type hypersensitivity (DTH) in mice (Crowle, 1975), was also regulated by suppressor T cells in mice injected i.v. with 10^9 horse red blood cells (HRBC) (Ramshaw, Bretscher & Parish, 1976) and 10^9 sheep red blood cells (SRBC) (Liew, 1977). These suppressor T cells may inhibit the induction of DTH (Phanuphak *et al.*, 1974), the expression of DTH (Zembala & Asherson, 1973) or both (Ramshaw *et al.*, 1976; Liew, 1977). On the other hand, antibody or antigen–antibody complexes were shown to inhibit both the induction and the expression of FPR to SRBC in mice (Mackaness, Lagrange, Miller & Ishibashi, 1974). Under these circumstances, it may be

preferable to investigate the regulatory mechanism of DTH in a FPR system where no antibody responses are induced.

In previous work, we prepared a derivative of lysozyme (Lys), D.Lys, by conjugating Lys with dodecanoic acid, which selectively induced FPR to Lys with no antibody response in mice (Kojima, Sugimoto & Egashira, 1976). The FPR induced with D.Lys was enhanced by treatment with cyclophosphamide (CY), suggesting the participation of suppressor cells in the FPR, as CY had been shown to eliminate the precursors of suppressor cells (Turk, Parker & Poulter, 1972; Polak & Turk, 1974; Gill & Liew, 1978).

In the experiments presented here, the regulation of the expression of DTH was investigated in more detail in the FPR system in the absence of antibody. The cellular nature and transient appearance of suppressor cells involved in the regulation of the FPR is described.

MATERIALS AND METHODS

Animals

Female ddY/S mice from 6 to 8 weeks old were used in all experiments except those described in Fig. 2.

Antigens

Six-times crystallized hen egg white lysozyme (Lys) was obtained from Seikagaku Kogyo Co., Tokyo. Dodecanoic acid-conjugated lysozyme (D.Lys) was prepared as described previously (Kojima *et al.*, 1976) according to the method of Coon & Hunter (1973). Alpha-lactalbumin (LA) (Sigma Co., St Louis) was dissolved in phosphate buffered saline (pH 7·5) and then centrifuged at 100,000 ***g*** for 60 min to remove insoluble materials.

Sensitization

Groups of six to eight mice were immunized subcutaneously (s.c.) with 100 μg D.Lys in saline, which induced FPR to Lys without antibody responses (Kojima *et al.*, 1976). For the production of high levels of FPR, mice were injected intraperitoneally with 300 mg/kg of cyclophosphamide (Endoxan, Shionogi Co., Osaka) 3 days before the immunization. A state of unresponsiveness for FPR was induced by priming s.c. with 10 mg Lys 14 days previously.

Cell transfer

All cell suspensions were prepared in balanced salt solution (BSS, pH 7·2) (Golub, Mishell, Weigle & Dutton, 1968). Spleen cells were prepared by gently teasing the spleen with forceps. The thymus was scissored and disrupted to a single cell suspension by pipetting gently with capillary pipette. The cells were passed through a stainless mesh and washed three times with minimum essential medium (MEM, Nissui Co., Tokyo). Five to 10×10^7 spleen cells or 2×10^7 thymus cells were transferred intravenously into normal mice or immune mice which had been injected with CY and immunized with D.Lys 7 days previously. The recipient mice were challenged for DTH in the footpad within 2 h after the cell transfer and their footpad swelling was determined 24 h later.

Anti-θ treatment of spleen cells

Anti-brain-associated θ antiserum was raised in rabbits according to the method of Golub (1971). The anti-θ antiserum was absorbed with liver cells, bone marrow cells, and red blood cells. At a 1/32 dilution the absorbed anti-θ antiserum killed more than 90% of thymus cells and about 30% of spleen cells by the trypan blue exclusion test. For the depletion of T cells from spleen cell suspensions, cells (2×10^8 cells/ml) were mixed with an equal volume of a 1/2 dilution of anti-θ antiserum and incubated at 4° for 60 min. After washing with BSS, the cells were suspended in a 1/3 dilution of agarose-absorbed guinea-pig serum (Cohen & Schlesinger, 1970) and incubated at 37° for 30 min. They were washed with BSS and resuspended in MEM for cell transfer.

Footpad test for DTH

DTH was determined as footpad swelling as described previously (Sugimoto, Kojima, Yaginuma & Egashira, 1975). Seven days after immunization with D.Lys, mice received eliciting injection with 2·5 μg Lys emulsified in Freund's incomplete adjuvant into the right footpad and with an emulsion without the antigen into the left footpad as a control. Footpad thickness was measured 24 h after the eliciting injection and specific footpad swelling was determined by subtracting the thickness of the left footpad from that of the right. Normal mice were challenged only for the non-specific footpad swelling as a negative control.

Serum antibody assay

Hyperimmune serum was raised in mice by three s.c. injections of 100 μg Lys in Freund's complete adjuvant (CFA) at 2-week intervals. The mice were bled 2 weeks after the last injection. Serum from unresponsive mice

were taken 2 weeks after priming with 10 mg Lys. Antibody titres to Lys in the heat-inactivated sera were determined by passive haemagglutination using SRBC coupled with Lys by the method of Johnson, Smith & Hall (1968).

RESULTS

Induction of antigen-specific suppression of FPR

Effect of priming dose of Lys on suppression of FPR was investigated. Mice were primed s.c. with various doses (10 μg to 10 mg) of Lys and 14 days later immunized with 100 μg D.Lys in saline. FPR was elicited with Lys 7 days after the immunization (Fig. 1). Priming with 10 μg and 100 μg Lys did not affect FPR induced with D.Lys. With increased priming doses of Lys, however, mice produced depressed FPR (unresponsiveness) to a subsequent immunization with D.Lys, and FPR was markedly reduced by priming with 10 mg Lys. Thus, in the following experiments, mice primed s.c. with 10 mg Lys were used 14 days later as donors of Lys-primed suppressors for FPR.

The unresponsiveness was antigen-specific (Table 1): Mice primed with 10 mg Lys were rendered unresponsive to subsequent immunization with D.Lys or Lys in CFA but responsive to the immunization with LA in CFA, whereas mice primed with 10 mg LA were unresponsive to LA but not to Lys.

Suppression of the expression of FPR by thymus-derived cells from unresponsive mice

Suppressive activity of spleen or thymus cells from unresponsive mice was assessed in cell transfer experiments into immune mice. Mice injected with CY on day -3 and immunized with D.Lys on day 0 produced high levels of FPR to Lys on day 7 (D.Lys-immune mice). These mice received the injection of spleen cells (1×10^8) or thymus cells (2×10^7) from syngeneic unresponsive donors which had been primed with 10 mg Lys 14 days previously. The recipient mice were challenged with Lys into the footpads within 2 h after the cell transfer, and their FPR was determined 24 h later (Fig. 2). FPR in D.Lys-immune mice was not affected by the transfer of spleen cells from normal mice; the transfer of spleen cells from unresponsive mice suppressed FPR in the immune recipients. Thymus cells (2×10^7) from unresponsive mice were as effective as spleen cells (1×10^8) from the same donors in suppression of FPR.

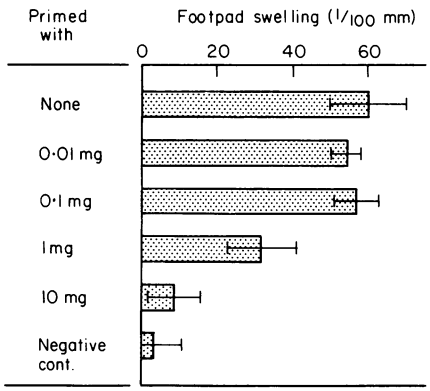

Figure 1. Effect of priming doses of Lys on FPR. Mice were primed s.c. with various doses (10 μg to 10 mg) of Lys and 14 days later immunized s.c. with 100 μg D.Lys. FPR to Lys were elicited 7 days after the immunization. Normal mice were challenged only for FPR as a negative control.

Table 1. Antigen-specific suppression of FPR

	Footpad swelling (±S.E.) (1/100 mm)		
Primed with	D.Lys	Lys in CFA	LA in CFA
None	62·4±12·1	84·9±11·6	70·3±9·6
Lys	12·7± 9·8	26·6± 8·9	72·3±5·3
LA	64·5± 7·1	80·0±11·9	35·1±7·7
Negative cont.*	3·3± 7·8	—	—

Mice were primed s.c. with 10 mg Lys or LA and 14 days later immunized with either 100 μg D.Lys in saline, 100 μg Lys in CFA, or 100 μg LA in CFA. FPR was elicited 7 days later in mice immunized with D.Lys, and 14 days later in mice immunized with Lys in CFA or LA in CFA.

* Normal mice received only eliciting injection into footpad.

The T-cell dependence of the suppression of FPR is shown in Fig. 3. Spleen cells from unresponsive mice were treated with anti-θ antiserum and complement and then 1×10^8 viable cells were transferred into D.Lys-immune mice. This treatment abolished the ability of the spleen cells to suppress the expression of FPR in the immune recipients.

These results suggest that thymus-derived cells (T cells) are responsible for suppression of the expression of FPR.

Antigen specificity of suppressor cells

The antigen specificity of the inhibition of expression of FPR by suppressor cells was investigated (Fig. 4).

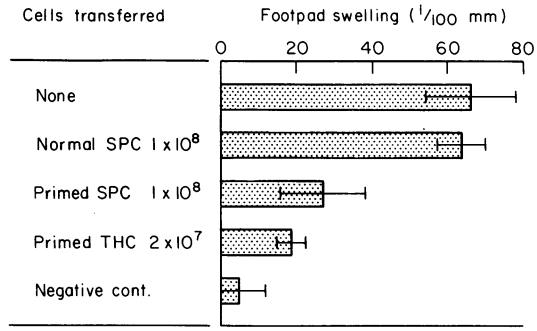

Figure 2. Suppression of the expression of FPR in immune recipients by the spleen and thymus cells from unresponsive mice. Spleen cells (1×10^8) and thymus cells (2×10^7) from DDY mice primed 14 days previously with 10 mg Lys were transferred into syngeneic mice which had been injected with CY (300 mg/kg) and immunized with D.Lys 7 days previously. The recipient mice were challenged for FPR immediately after the cell transfer. SPC, spleen cells; THC, thymus cells.

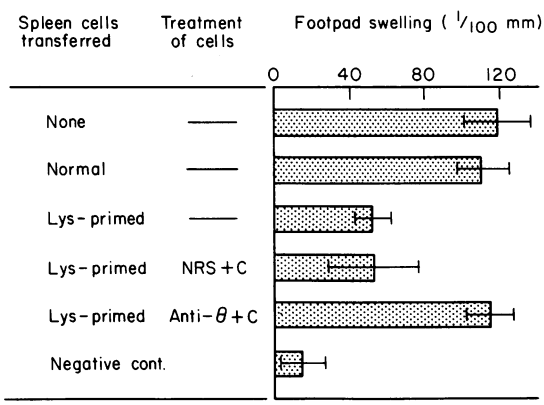

Figure 3. Effect of treatment with anti-θ antiserum and complement on suppressive spleen cells. Suppressive spleen cells from mice primed 14 days previously with 10 mg Lys were treated with either rabbit anti-θ antiserum or normal rabbit serum (NRS) plus complement (C), and then 1×10^8 viable cells were transferred into CY-treated D.Lys-immune recipients. The recipient mice were challenged for FPR immediately after the cell transfer.

Spleen cells (1×10^8) from mice primed 14 days previously with 10 mg Lys or LA were transferred into D.Lys-immune mice (Fig. 4A), or LA-immune mice (Fig. 4B). FPR in the D.Lys-immune mice was depressed by the transfer of Lys-primed spleen cells but not of LA-primed spleen cells. In contrast, FPR in LA-immune mice was not affected by the transfer of Lys-primed spleen cells but suppressed by LA-primed spleen cells. These results suggest that suppressor cells

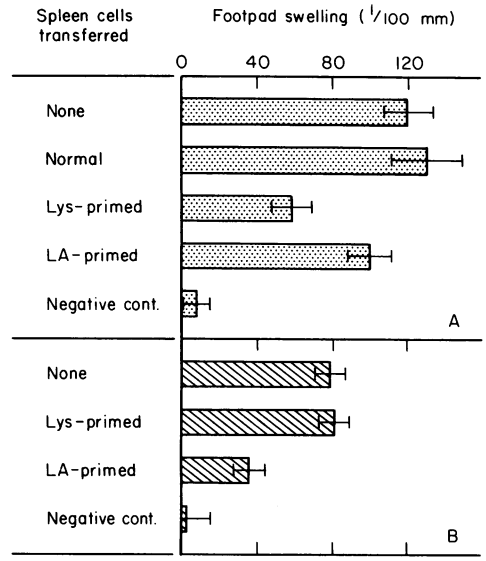

Figure 4. Antigen specificity of suppressor cells. Suppressive spleen cells (1×10^8) taken 14 days after priming with 10 mg Lys or LA were transferred into CY-treated D.Lys-immune mice (A), or LA-immune mice injected with 100 μg LA in CFA 14 days previously (B). FPR was elicited immediately after the cell transfer.

in the expression of FPR express their function antigen-specifically.

Inability of antibody to suppress the expression of FPR

The role of serum antibody in suppression of the expression of FPR was investigated. D.Lys-immune mice were given either normal serum, hyperimmune serum, or serum from unresponsive mice immediately before elicitation of FPR (Table 2). Serum from unresponsive mice (anti-Lys titre: 2^2, in this experiment) did not affect the levels of FPR in the immune mice. Furthermore, hyperimmune serum (anti-Lys titre: 2^9) failed to affect the levels of FPR, although the serum could transfer high anti-Lys antibody titres into the recipient mice. This shows that serum antibody does not play any role in suppression of the expression of FPR.

Depression of passive transfer of FPR

In the following experiment, whether the suppressor cells can inhibit the function of immune cells responsible for FPR was investigated in a passive transfer system of FPR into normal mice. Spleen cells were taken from D.Lys-immune mice 7 days after immuni-

Table 2. Inability of serum to suppress the expression of FPR in D.Lys-immune mice

Serum transferred (0·2 ml)	Footpad swelling (±S.E.) (1/100 mm)	Anti-Lys titre* (log_2)
None	169·0±17·8	0
Normal serum	156·1±18·3	0
Serum from unresponsive mice	167·7±26·1	0·2±0·1
Hyperimmune serum	159·8±27·9	4·7±0·4
Negative control†	6·6± 6·6	0

Mice injected with CY and immunized with D.Lys 7 days previously were given either normal serum, hyperimmune serum, or serum from mice primed 14 days previously with 10 mg Lys. FPR was immediately elicited in recipient mice.

* Anti-Lys antibody titres in recipient mice.

† Normal mice received only eliciting injection into footpad.

zation. The D.Lys-immune spleen cells (5×10^7) were transferred alone or in combination with Lys-primed spleen cells (5×10^7) from unresponsive mice into normal mice. The normal recipients were immediately challenged for DTH into footpad (Table 3). The D.Lys-immune spleen cells alone could passively transfer FPR into normal mice although their ability to transfer FPR was depressed by the addition of Lys-primed spleen cells from unresponsive mice.

Table 3. Depression of transfer of FPR by immune spleen cells into normal recipient mice

Spleen cells transferred		Footpad swelling (±S.E.) (1/100 mm)
D.Lys-immune	Lys-primed	
5×10^7	None	40·7±8·2
5×10^7	5×10^7	11·1±8·9
None	None	6·3±6·7

D.Lys-immune spleen cells were taken 7 days after immunization from mice injected with CY and immunized with D.Lys. Suppressive spleen cells were taken from mice primed 14 days previously with 10 mg Lys. The immune spleen cells were transferred alone or in combination with suppressive spleen cells into normal mice. The recipient mice were immediately challenged into the footpads and their 24 h footpad swelling was determined.

Persistence of unresponsiveness and transient appearance of suppressor cells

The kinetics of unresponsiveness was compared with that of development of suppressor cells in the thymus and spleen in unresponsive mice.

Mice were primed with 10 mg Lys and immunized with D.Lys 0, 1, 2, 3, 5 and 7 weeks after the priming. FPR was elicited 7 days after the immunization (Fig. 5). Mice primed with 10 mg Lys at the same time of the immunization were rendered unresponsive for FPR on day 7. This unresponsiveness was also observed in mice primed with 10 mg Lys 1–7 weeks earlier.

The kinetics of development of suppressor cells in both the spleen and the thymus in unresponsive mice were investigated in the following experiments. Spleen cells (1×10^8) and thymus cells (2×10^7) were taken from unresponsive mice 1–7 weeks after unresponsiveness induction and transferred into D.Lys-immune mice. The immune recipients were challenged for DTH into the footpads immediately after the cell transfer (Fig. 6). Suppressor cells appeared 2 weeks after unresponsiveness induction in both the spleen and the thymus. Both spleen and thymus cells, however, from mice 3–7 weeks after unresponsiveness induction lost their ability to suppress FPR, although donor mice remained fully unresponsive.

These results show that thymus and spleen cells of unresponsive mice are active in suppressing the expression of FPR only for a definite period of the long-lasting unresponsiveness phase.

DISCUSSION

In the present experiments, the properties of suppressor cells in the expression of DTH in mice were investi-

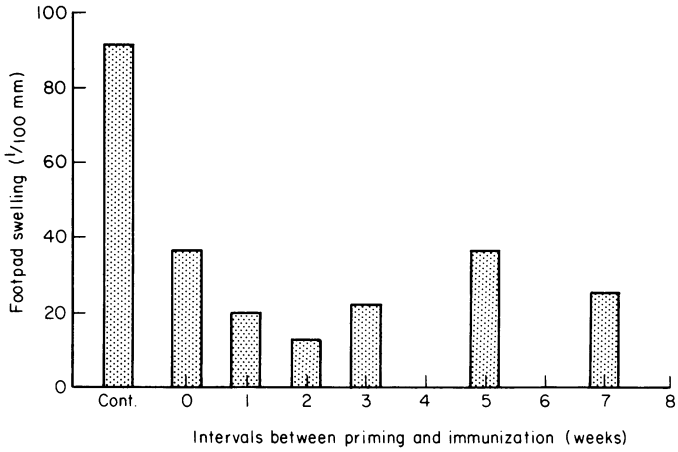

Figure 5. Kinetics of unresponsiveness induction. Mice were primed s.c. with 10 mg Lys and various times later immunized with D.Lys. FPR was elicited 7 days after the immunization. Control mice were not primed with 10 mg Lys but immunized with D.Lys.

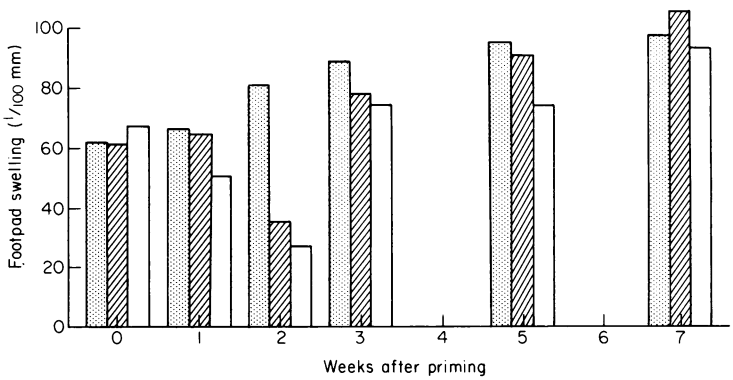

Figure 6. Kinetics of the development of suppressor cells in the spleen and thymus. Mice were primed s.c. with 10 mg Lys. Various times after the priming, spleen (1×10^8) and thymus cells (2×10^7) were taken and transferred into mice which had been injected with CY and immunized with D.Lys 7 days previously. FPR was elicited immediately after cell transfer. Control mice received no cells. Week '0' indicates mice receiving transfer of normal cells. Stippled column, control mice; hatched column, spleen cell recipients; open column, thymus cell recipients.

gated in a FPR system where no antibody responses were produced with use of a chemically modified derivative of Lys, D.Lys, as an immunizing antigen (Kojima *et al.*, 1976). The results show that mice rendered unresponsive by priming s.c. with a single dose of Lys, produced suppressor cells which suppressed the expression of FPR in D.Lys-immune mice and also inhibited the transfer of FPR by the immune lymphoid cells into normal mice (Fig. 2, Table 3). These suppressor cells are antigen-specific as suppressor cells induced with Lys inhibited FPR in D.Lys-immune mice but not FPR in LA-immune mice and *vice versa* (Fig. 4). The suppressor cells are T cell in nature, as they were sensitive to the treatment with anti-θ and complement (Fig. 3), and this was supported by experiments where 2×10^7 thymus cells displayed similar extents of suppressive effects as did 1×10^8 spleen cells (Fig. 2). The presence of suppressor T cells in the thymus is in

keeping with the earlier observations on suppressor T cells in the induction of skin reaction in rat (Ha & Waksman, 1973) and FPR in mice (Liew, 1977). This is not consistent, however, with the reports by Asherson & Zembala (1974) and Claman, Miller & Moorhead (1976) who have described that tolerance of contact sensitivity could not be transferred by thymus cells from tolerant donors, suggesting the suppressor T cells have to be peripheral T lymphocytes. Suppressive role of thymus cells in the expression of FPR to D.Lys is described in Kojima, Tamura & Egashira (1979).

The suppression of the expression of FPR to D.Lys is not likely to be due to serum antibody, as has been reported by Mackaness *et al.* (1974). First, only low levels of serum antibody were produced in unresponsive mice, and in many cases antibody levels were very low or not detectable. Secondly, the transfer of serum from unresponsive mice or hyperimmune serum failed to suppress the FPR, although the hyperimmune serum could transfer significant levels of antibody into recipient mice (Table 2). This is consistent with the results by Easmon & Glynn (1977) who have found that immune serum had no effect on the expression of FPR to staphylococci. The possibility may be argued, however, that suppressor T cells were effective in the influence of excess antigen or antigen–antibody complexes, because unresponsiveness of donor mice was induced by the injection with a high dose of antigen. This seems not to be the case. Transfer of suppressive activity in both the spleen and the thymus was not detected 1 week after the injection but detected 2 weeks after the injection (Fig. 6). The transferred cells were washed well. Moreover, D.Lys induced no detectable antibody in recipient mice (Kojima *et al.*, 1976), and antigen–antibody complexes could not be formed in them even if antigen was transferred. Furthermore, extract from the thymus and spleen of unresponsive mice had no suppressive effect (Kojima *et al.*, 1979). Nevertheless, it is not ruled out that the suppressive activity in the serum, as has been reported by Mackaness *et al.* (1974), may be mediated by factor(s) released by suppressor T cells. The role of a soluble suppressor T cells factor for the expression of FPR in the present system is described in Kojima *et al.* (1979).

Kinetics of unresponsiveness showed discrepancy between the persistence of unresponsiveness and the transient appearance of suppressor cells . Unresponsiveness induced by priming s.c. with Lys persisted at least up to 7 weeks after the priming (Fig. 5). In contrast, suppressor cells appeared in the thymus only 2 weeks after unresponsiveness induction and were no longer detectable 3–7 weeks later in mice which remained fully unresponsive to a subsequent immunization with D.Lys (Fig. 6). This loss of suppressor cells in the thymus is not likely to be due to their migration out from the thymus toward the spleen; in the spleen suppressor cells were also detected 2 weeks after unresponsiveness induction and disappeared thereafter (Fig. 6). The transient appearance of suppressor T cells in our system is consistent with that of suppressor T cells involved in the induction of DNFB contact sensitivity (Miller, Sy & Claman, 1977), FPR to SRBC (Liew, 1977), and skin reaction to bovine γ-globulin in rats (Ha & Waksman, 1973). These reports have shown that suppressive activity in spleen and lymph node cells of unresponsive mice was detectable only for a definite period of time after unresponsiveness induction, while unresponsiveness in donor mice persisted for a much longer time. Although it is difficult to understand why detectable suppressive activity disappears entirely, one explanation is inhibition of effector T cell clones as has been suggested by Miller *et al.* (1977). Another is that a further type of cell maintains suppression in unresponsive mice. In support of this view is the finding by Zembala & Asherson (1974) who have shown that suppressive activity of suppressor T cells was mediated by macrophages. It may be also possible that suppressor T cells are active only for a short period of time but retain a long-lasting immunological memory such as memory suppressor T cells in antibody responses recently described by Loblay, Pritchard-Brisco & Basten (1978). Further experiments are required to examine these possibilities.

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REFERENCES

ASHERSON G.L. & ZEMBALA M. (1974) Suppression of contact sensitivity by T cells in the mouse. I. Demonstration that suppressor cells act on the effector stage of contact sensitivity; and their induction following *in vitro* exposure to antigen. *Proc. R. Soc. Lond. B.* **187**, 329.

CLAMAN H.N., MILLER S.D. & MOORHEAD J.W. (1976) Tolerance: two pathways of negative immunoregulation in contact sensitivity to DNFB. *Cold Spring Harbor Symp. Quant. Biol.* **41,** 105.

COHEN A. & SCHLESINGER M. (1970) Absorption of guinea pig serum with agar. A method for elimination of its cytotoxicity for murine thymus cells. *Transplantation,* **10,** 130.

COON J. & HUNTER R. (1973) Selective induction of delayed hypersensitivity by a lipid conjugated protein antigen which is localized in thymus dependent lymphoid tissue. *J. Immunol.* **110,** 183.

CROWLE A.J. (1975) Delayed hypersensitivity in the mouse. *Advanc. Immunol.* 20, 197.

EASMON C.S.F. & GLYNN A.A. (1977) Effect of cyclophosphamide on delayed hypersensitivity to *Staphylococcus aureus* in mice. *Immunology,* **33,** 767.

GILL H.K. & LIEW F.Y. (1978) Regulation of delayed-type hypersensitivity. III. Effect of cyclophosphamide on the suppressor cells for delayed-type hypersensitivity to sheep erythrocytes in mice. *Europ. J. Immunol.* **8,** 172.

GOLUB E.S. (1971) Brain-associated θ antigen: Reactivity of rabbit anti-mouse brain with mouse lymphoid cells. *Cell. Immunol.* **2,** 353.

GOLUB E.S., MISHELL R.I., WEIGLE W.O. & DUTTON R.W. (1968) A modification of the hemolytic plaque assay for use with protein antigens. *J. Immunol.* **100,** 133.

HA T.-Y. & WAKSMAN B.H. (1973) Role of the thymus in tolerance. X. 'Suppressor' activity of antigen-stimulated rat thymocytes transferred to normal recipients. *J. Immunol.* **110,** 1290.

JOHNSON H.M., SMITH B.G. & HALL H.E. (1968) Carbodiimide hemagglutination: a study of some of the variables of the coupling reaction. *Int. Archs Allergy,* **33,** 511.

KOJIMA A., SUGIMOTO M. & EGASHIRA Y. (1976) Immunogenicity of lysozyme derivatives lipid-conjugated to various degrees in mice treated with and without cyclophosphamide: dissociation of delayed-type hypersensitivity and helper function. *Japan. J. med. Sci. Biol.* **29,** 323.

KOJIMA A., TAMURA S.-I. & EGASHIRA Y. (1979) Regulatory role of suppressor T cells in the expression of delayed-type hypersensitivity in mice. II. Soluble factor from thymic suppressor cells stimulated with antigen *in vitro* and its possible interaction with macrophages. *Immunology,* **37,** 577.

LIEW F.Y. (1977) Regulation of delayed-type hypersensitivity. I. T suppressor cells for delayed-type hypersensitivity to sheep erythrocytes in mice. *Europ. J. Immunol.* **7,** 714.

LOBLAY R.H., PRITCHARD-BRISCOE H. & BASTEN A. (1978) Suppressor T-cell memory. *Nature (Lond.),* **272,** 620.

MACKANESS G.B., LAGRANGE P.H., MILLER T.E. & ISHIBASHI T. (1974) Feedback inhibition of specifically sensitized lymphocytes. *J. exp., Med.* **139,** 543.

MILLER S.D., SY M.-S. & CLAMAN H.M. (1977) The induction of hapten-specific T cell tolerance using hapten-modified lymphoid membranes. II. Relative roles of suppressor T cells and clone inhibition in the tolerant state. *Europ. J. Immunol.* **7,** 165.

PHANUPHAK P., MOORHEAD J.W. & CLAMAN H.N. (1974) Tolerance and contact sensitivity to DNFB in mice. III. Transfer of tolerance with 'suppressor T cells'. *J. Immunol.* **113,** 1230.

POLAK L. & TURK J.L. (1974) Reversal of immunological tolerance by cyclophosphamide through inhibition of suppressor cell activity. *Nature (Lond.),* **249,** 654.

RAMSHAW I.A., BRETSCHER P.A. & PARISH C.R. (1976) Regulation of the immune response. I. Suppression of delayed-type hypersensitivity by T cells from mice expressing humoral immunity. *Europ. J. Immunol.* **6,** 674.

SUGIMOTO M., KOJIMA A., YAGINUMA K. & EGASHIRA Y. (1975) Cell-mediated and humoral immunity in mice: cross reaction between lysozyme and S-carboxymethylated lysozyme studied by a modified footpad test. *Japan. J. med. Sci. Biol.* **28,** 23.

TURK J.L., PARKER D. & POULTER L.W. (1972) Functional aspects of the selective depletion of lymphoid tissue by cyclophosphamide. *Immunology,* **23,** 493.

ZEMBALA M. & ASHERSON G.L. (1973) Depression of the T cell phenomenon of contact sensitivity by T cells from unresponsive mice. *Nature (Lond.),* **244,** 227.