Immunology 1977 **32** 237

Cell-mediated and humoral immune responses in mice

IV. DIFFERENCE OF THE FUNCTIONAL CELL POPULATION BETWEEN HELPER ACTIVITY AND DELAYED-TYPE HYPERSENSITIVITY

Y. KATSURA, M. TAKAOKI, S. NISHIKAWA & S. MURAMATSU *Department of Bacteriology and Serology, Chest Disease Research Institute and Department of Zoology, Faculty of Science, Kyoto University, Kyoto, Japan*

Received 5 *March* 1976; *accepted for publication* 23 *July* 1976

Summary. Helper activity in the anti-hapten antibody response was studied in mice in reference to the induction of delayed-type hypersensitivity (DTH) to the carrier protein. Mice were immunized either by an i.v. injection of alum-precipitated bovine serum albumin (AP-BSA) plus bacterial endotoxin or by a s.c. injection of BSA in Freund's complete adjuvant, the latter being effective in inducing DTH. The helper activity was estimated by the antibody response to the challenge with dinitrophenylated BSA (DNP-BSA) given at varying intervals after the injection of BSA. The results indicated that the helper activity was independent of DTH to the carrier protein, suggesting that these two activities, are mediated by different populations of functional cells.

A low dose of tolerogenic soluble BSA (sBSA) was sufficient to abrogate the helper activity in the response to DNP-BSA. In contrast, DTH to BSA was only partially depressed by the pretreatment with a low dose of sBSA and was completely depressed by a high dose. DTH reactivity in mice pretreated with a low dose of tolerogen and followed by the immunization with BSA in Freund's complete adjuvant was substantiated by the microscopic observation of mononuclear cell infiltration at the site of the test antigen injection. These results suggest that cells involved in the helper function and DTH may be derived from different precursors.

Correspondence: Dr Y. Katsura, Department of Bacteriology and Serology, Chest Disease Research Institute, Kyoto University, Kyoto, Japan.

INTRODUCTION

Antibody response and the delayed-type hypersensitivity (DTH) may be induced independently of each other. Tamura & Egashira (1975) showed that the i.p. injection of sheep erythrocytes into mice activated the helper function without the induction of delayed-type hypersensitivity (DTH). The s.c. injection was effective in generating both helper activity and DTH. Dennert & Lennox (1974) demonstrated the preferential activation of the helper function in mice by the i.p. injection of a low dose of irradiated or formaldehyde-treated mastocytoma P815 cells, in contrast to the development of the killer activity by the injection of live P815 cells through the same route. Nordin & Farrar (1974) and Dauphinee & Nordin (1974) revealed the defect in helper activity in allogeneic bone marrow chimera mice without the impairment of cell-mediated immunity. Differential generation of the helper function or DTH was also shown in rats using salmonella flagellin and its modified forms (Liew & Parish, 1974).

In spite of these findings, it seems still ambiguous as to whether the helper function and DTH are the responses of a single T-cell type or of different

T-cell subsets. If the latter is the case, there should be the following possibilities: that these different subsets originate from a common type of antigen-sensitive precursor or that they are derived from different precursors. The study through the tolerance induction may be a valid approach to the problem about precursor cells. In guinea-pigs, Borel, Fauconnet & Miescher (1966) showed that cells involved in DTH were more sensitive than those involved in antibody response to neonatal tolerization. However, the study by Silver & Benacerraf (1974) led us to the inverse conclusion, since they showed that the injection of deaggregated human gammaglobulin into adult guinea-pigs completely abrogated the helper activity while DTH was suppressed only partially.

The present study was performed in mice with a T-cell dependent antigen, bovine serum albumin, and presents evidence for the differential induction of helper activity and DTH after immunization, as well as the differential effect of tolerance induction. Results are discussed in reference to the origin of precursor cells.

MATERIALS AND METHODS

Animals

Male and female CBA/StMs mice (originally obtained from National Institute of Genetics, Mishima, Japan) were used. Mice were 7–10 weeks of age at the beginning of the treatment.

Antigens

Crystalline bovine serum albumin (BSA) (Miles Laboratories, Kankakee, Illinois, lot no. 19) was mainly used. Human gamma globulin (HGG) (Nutritional Biochemical Corporation, Cleveland, Ohio) was also used. Dinitrophenylated BSA (DNP-BSA) and dinitrophenylated HGG (DNP-HGG) were prepared according to the method of Little and Eisen (1967). Seventeen DNP groups were conjugated with one molecule of carrier proteins.

Tolerance induction

Soluble BSA (sBSA) deaggregated by ultracentrifugation was used as tolerogen (Katsura, 1972; Muramatsu, Amagai & Katsura, 1975). Low dose tolerance was induced by four i.v. injections of 0·1 mg sBSA once a week, and high dose tolerance induced by weekly injection of 0·1 mg sBSA on the first injection and 5 mg on the following three injections.

Immunization

Freund's complete adjuvant (FCA) was prepared by the addition of 10 mg of heat-killed *Mycobacterium tuberculosis* $H_{37}Rv$ (produced in our laboratory) in 1 ml of Freund's incomplete adjuvant (Difco Laboratories, Detroit, Michigan). A 1:1 mixture of the adjuvant and saline, with or without antigen (1 per cent), was converted into water-in-oil emulsion with a high speed laboratory mixer, and 0·2 ml of the emulsion was injected s.c. at a dorsal site.

Alum-precipitated BSA (AP-BSA) was prepared as described previously (Katsura, Nakano, Kohara & Uesaka, 1976a). The same procedure as for AP-BSA was used for the preparation of alum-precipitated DNP-BSA (AP-DNP-BSA) and alum-precipitated DNP-HGG (AP-DNP-HGG). The suspension of AP-BSA in saline was injected i.v. with bacterial endotoxin (ET) (lipopolysaccharide B, *E. coli* 0111:B4, Difco Laboratories, Detroit, Michigan). AP-DNP-BSA or AP-DNP-HGG was injected i.v. without endotoxin.

Footpad test

Procedures for the injection of test antigen and the measurement of the swelling were the same as in the previous paper (Katsura *et al.*, 1976a). The swellings at the 3rd and 48th h after the footpad injection of 0·01 mg of AP-BSA were regarded as Arthus-type and delayed-type reaction, respectively (Katsura, Inaba, Izumi & Uesaka, 1976b).

Plaque-forming cell assays

Antibody-forming cells were assessed as plaque-forming cells (PFC). The method of the conjugation of BSA on sheep red blood cells (SRBC) and procedures of the plaque assay on microslides were described elsewhere (Takaoki, Kawaguchi, Katsura & Muramatsu, 1976). Anti-DNP plaque-forming cells were assayed using trinitrophenylated SRBC (TNP-SRBC) as target cells (Rittenberg & Pratt, 1969). Anti-mouse immunoglobulin rabbit serum (anti-MIg) diluted 1:200 was used to develop indirect PFC. Anti-BSA indirect PFC include both IgM and IgG-forming cells (Takaoki *et al.*, 1976). Anti-DNP IgM PFC were detectable by the direct method. Indirect PFC against DNP, do not represent the total of anti-DNP IgG- and IgM-forming cells, since anti-MIg suppressed partially the development of anti-DNP IgM PFC.

Table 1. Nonspecific stimulation of anti-DNP antibody response

Group	Treatment*	Tested at (weeks)	Anti-DNP PFC per spleen		Anti-SRBC indirect PFC per spleen
			Direct	Indirect	
I	not treated		1070 (1·32)†	850 (1·16)	< 100
II	ET	2	2950 (1·10)	950 (1·27)	n.d.
III	FCA	2	3500 (1·07)	1970 (1·19)	n.d.
IV	AP-BSA plus ET	2	5580 (1·14)	3270 (1·41)	720 (1·38)
V	FCA-BSA	2	4890 (1·22)	2380 (1·20)	470 (1·27)
VI	AP-BSA plus ET	8	2690 (1·17)	900 (1·45)	n.d.
VII	FCA-BSA	8	2000 (1·27)	690 (1·64)	n.d.

n.d. = Not determined.

* ET i.v. injection of 0·01 mg of ET; FCA, s.c. injection of FCA; AP-BSA plus ET, i.v. injection of 0·1 mg of AP-BSA and 0·01 mg of ET; FCA-BSA, s.c. injection of 1 mg of BSA in FCA. Each group comprised four to five mice.

† Geometrical mean. Values in parentheses represent standard errors.

Histology

Feet of mice were fixed with 10 per cent formalin, and their skins were imbedded in paraffin. Sections were made and stained with haematoxylin and eosin.

Anti-thymocyte serum (ATS)

Rabbit anti-mouse thymocyte serum prepared as described previously (Katsura *et al.*, 1976b) was injected into mice to eliminate the thymus-derived cells.

RESULTS

Development of helper activity without the induction of DTH

Nonspecific stimulation

In the study of anti-DNP or anti-TNP response, a relatively high background level of PFC and the elevation of the background after certain nonspecific stimulations, such as adjuvants or unrelated antigen, should be taken into consideration.

Groups of mice were given an i.v. injection of 0·01 mg of ET, a s.c. injection of FCA, and i.v. injection of 0·1 mg of AP-BSA plus 0·01 mg of ET (AP-BSA plus ET), or a s.c. injection of 1 mg of BSA in FCA (FCA-BSA). Another group was left untreated. Two weeks, or in some groups 8 weeks, after the treatment, mice were killed and their spleens were assayed for PFC. As shown in Table 1, the background level of anti-DNP PFC in normal mice was more than ten times as high as that of anti-SRBC (group I). Injection of adjuvants, ET or FCA, caused an increase in the number of anti-DNP PFC, the level in direct PFC 2 weeks after the injection being about three times higher than that in normal mice. Further increase in the background level was seen in mice given BSA with adjuvants 2 weeks previously. High levels of anti-DNP antibody production were observed in nonspecifically stimulated mice compared with normal mice, even 8 weeks after the injection. In any case, the injection of AP-BSA plus ET or FCA-BSA resulted in the nonspecific augmentation of the anti-DNP background level.

Specificity of the carrier effect

Mice were primed with a s.c. injection of either FCA-BSA or FCA-HGG, and challenged 2 weeks later with DNP-BSA or DNP-HGG. Numbers of anti-DNP PFC in spleen at day 4 after the challenge are shown in Table 2. It is indicated that the helper activity in the anti-DNP antibody response was specific to the carrier used for priming. Table 2 also shows that the pretreatment with anti-BSA antiserum did not enhance the response to DNP-BSA.

Kinetics of the helper activation

In the previous paper (Katsura *et al.*, 1976b), it was

Table 2. Specificity of the carrier effect

Group	Treatment*	Challenge†	Anti-DNP PFC per spleen‡ Direct	Indirect
I	FCA-BSA	DNP-BSA	13,200 (1·07)§	13,700 (1·39)
II	FCA-BSA	DNP-HGG	3050 (1·12)	1250 (1·36)
III	FCA-HGG	DNP-BSA	2560 (1·23)	1410 (1·22)
IV	FCA-HGG	DNP-HGG	14,700 (1·52)	10,000 (1·25)
V	anti-BSA	DNP-BSA	2430 (1·09)	1120 (1·17)
VI	NMS	DNP-BSA	2100 (1·31)	1660 (1·02)

* FCA-BSA, s.c. injection of 1 mg of BSA in FCA; FCA-HGG, s.c. injection of 1 mg of HGG in FCA; anti-BSA, i.v. injection of 1 ml of antiserum obtained from mice given s.c. injection of FCA-BSA 3 weeks previously; NMS, i.v. injection of 1 ml of normal mouse serum. Each group comprised four mice.

† In groups I–IV, mice were challenged i.v. either with 0·1 mg of AP-DNP_{17}-BSA (DNP-BSA) or with 0·1 mg of AP-DNP_{17}-HGG (DNP-HGG) 2 weeks after the treatment. In groups V and VI, mice were challenged with DNP-BSA immediately after the treatment.

‡ Assayed 4 days after the challenge.

§ Geometrical mean. Values in parentheses represent s.e.

Table 3. Anti-DNP antibody response in spleen and in inguinal lymph nodes of mice primed either with AP-BSA plus ET or FCA-BSA

Group	Treatment*	Challenge†	Organ	Anti-DNP PFC‡ Direct	Indirect	Anti-BSA‡ indirect PFC
I	AP-BSA plus ET	DNP-BSA	Spleen	14,300 (1·05)§	13,500 (1·10)	12,600 (1·29)
			Lymph nodes¶	< 10	< 10	< 10
II	FCA-BSA	DNP-BSA	Spleen	15,500 (1·20)	12,700 (1·38)	14,800 (1·10)
			Lymph nodes¶	20	< 10	1000

* AP-BSA plus ET, i.v. injection of 0·1 mg of AP-BSA and 0·01 mg of ET; FCA-BSA, s.c. injection of 1 mg of BSA in FCA. Each group comprised four mice.

† Intravenous injection of 0·1 mg of AP-DNP-BSA.

‡ Assayed 6 days after the challenge.

§ Geometrical mean. Values in parentheses represent s.e.

¶ Pooled lymph node cells were subjected to PFC assay. Then, the value represents the arithmetical mean.

shown that FCA-BSA was effective in inducing both DTH and antibody response and AP-BSA plus ET induced antibody response alone. This seems to suggest that the helper cell in antibody response and the effector cell in DTH may develop independently of each other. The present experiment was undertaken to confirm this by the employment of hapten-carrier conjugate antigen.

In the early stage after the injection of FCA-BSA, draining lymph nodes were found to be the main site of anti-BSA antibody production (Katsura *et al.* 1976b). Thus, anti-DNP PFC response in inguinal lymph nodes were compared with that in the spleen. Mice were primed either with AP-BSA plus ET or with FCA-BSA, and challenged 2 weeks later with an i.v. injection of 0·1 mg of AP-DNP-BSA. Six days after the challenge, the numbers of anti-DNP and anti-BSA PFC in spleen and lymph

nodes were determined. As shown in Table 3, the number of anti-DNP PFC in lymph nodes was negligibly small irrespective of the route of the primary immunization with BSA. Table 3 also indicates that at the 2nd week after the priming, the helper-activity in the spleen of mice given FCA-BSA was comparable to that of mice given AP-BSA plus ET.

Experiments were performed to compare the kinetics of helper activity and that of DTH. Mice were immunized either with AP-BSA plus ET or with FCA-BSA. They were challenged 2, 8 or 12 weeks later with an i.v. injection of 0·1 mg of AP-DNP-BSA to estimate the helper activity. Four days after the challenge, direct and indirect PFC against DNP in the spleen were determined. On the other hand, the footpad test was performed in separate groups to estimate the level of DTH 2, 8 and 12 weeks after immunization. Results are shown in Table 4 together with those of several control groups. Nonspecific priming by ET or FCA was observed also in this experiment as in the case shown in

Table 4. Priming of helper activity by either i.v. injection of AP-BSA plus ET or s.c. injection of FCA-BSA

Group	Treatment*	Interval (weeks) between treatment and challenge	Anti-DNP PFC per spleen†		Footpad swelling (48 h)‡
			Direct	Indirect	
I	None	0	2630 (1·17)§	1020 (1·38)	0·31±0·19
II	ET	2	4070 (1·14)	2750 (1·09)	n.d.
III	FCA	2	4680 (1·23)	2750 (1·18)	n.d.
IV	AP-BSA plus ET	2	16,000 (1·02)	13,700 (1·19)	0·50±0·31
V	AP-BSA plus ET	2 (ATS)¶	5280 (1·20)	3180 (1·23)	n.d.
VI	FCA-BSA	2	18,300 (1·23)	16,300 (1·26)	2·80±0·20
VII	AP-BSA plus ET	8	10,200 (1·04)	6640 (1·15)	0·00±0·12
VIII	FCA-BSA	8	4140 (1·23)	4170 (1·17)	n.d.
IX	AP-BSA plus ET	12	5890 (1·26)	3770 (1·20)	0·50±0·25
X	FCA-BSA	12	4360 (1·14)	2510 (1·12)	2·60±0·56

n.d. = Not determined.

* ET i.v. injection of 0·01 mg of ET; FCA, s.c. injection of FCA; AP-BSA plus ET, i.v. injection of 0·1 mg of AP-BSA and 0·01 mg of ET; FCA-BSA, s.c. injection of 1 mg of BSA in FCA. Each group comprised four to six mice.

† Assayed 4 days after the i.v. injection of 0·1 mg of AP-DNP-BSA.

‡ Data obtained in separate groups of mice. The swelling was indicated by the unit of one-tenth mm. Arithmetic mean of four mice ±1 s.e.

§ Geometrical mean. Values in parentheses represent s.e.

¶ Subcutaneous injection of 0·25 ml of ATS 2 days before challenge followed by the i.v. injection of 0·25 ml of ATS 1 h before challenge.

Table 5. Effect of tolerance induction to the carrier on the antibody response to the hapten

Group*	Tolerance induction	Carrier immunization (FCA-BSA)†	Challenge (DNP-BSA)‡	Anti-DNP PFC per spleen§	
				Direct	Indirect
I	Low dose	+	+	2340 (1·15)¶	1600 (1·20)
II	High dose	+	+	3020 (1·18)	1740 (1·29)
III	None	+	+	21,900 (1·35)	17,600 (1·48)
IV	None	−	+	1100 (1·31)	770 (1·45)

* Each group comprised five mice.

† Subcutaneous injection of 1 mg of BSA in FCA 1 week after the last injection of tolerogen.

‡ Intravenous injection of 0·1 mg of AP-DNP-BSA 3 weeks after the carrier immunization.

§ Assayed 4 days after the challenge.

¶ Geomtrical mean. Values in parentheses represent s.e.

Table 1. Although both AP-BSA plus ET and FCA-BSA were capable of generating helper cells, the duration of the helper activity was longer in mice primed with the former than in those with the latter. Since DTH to BSA was not detectable in mice primed with AP-BSA plus ET, it seems plausible that the level of DTH has no essential influence on the helper activity. Abrogation of the helper function by the injection of ATS (group V) suggested that the helper activity was carried on a recirculating cell which was probably a T cell.

Investigation by means of tolerance induction

The above experiments seemed to indicate that T cells participating in antibody response were different from those involved in DTH. The problem to be elucidated is whether antigen-sensitive precursor cells of these two T-cell populations are common or not. The analysis through the induction of tolerance to BSA may be expected to provide some answers to this question.

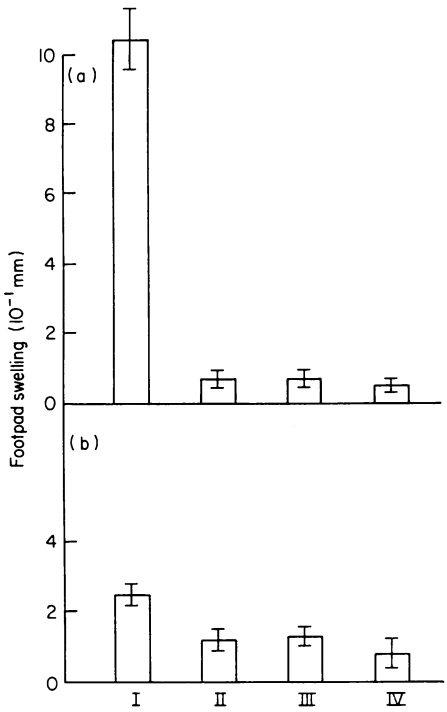

Figure 1. (a) Arthus (3 h) and (b) delayed (48 h) footpad reactions in tolerance-induced mice. Positive control mice (I) received a s.c. injection of 1 mg of BSA in FCA (FCA-BSA) without the induction of tolerance. 'Low dose' tolerant group (II) and 'high dose' tolerant group (III) were given FCA-BSA 1 week after the last injection of tolerogen, and negative control group (IV) received neither tolerogen nor FCA-BSA. The footpad test was performed with 0·01 mg of AP-BSA 3 weeks after the injection of FCA-BSA. Each column and each vertical bar represents, respectively, the arithmetical mean value of five mice and the standard error.

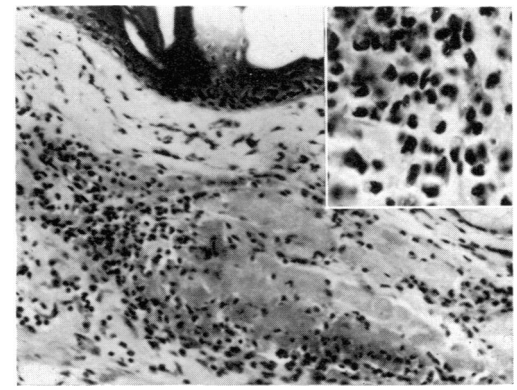

Figure 2. Histological appearance of the footpad of low dose tolerant mice. Experimental protocol was described in the legend of Fig. 1. The section was prepared 48 h after the injection of test antigen. Note the infiltration of mononuclear cells. (Haematoxylin and eosin, × 385). Morphological details of infiltrating cells are shown in high magnification (× 960) in the right upper corner.

Figure 3. Histological appearance of the footpad of high dose tolerant mice. Experimental protocol was described in the legend of Fig. 1. The section was prepared 48 h after the injection of test antigen. Note the absence of the infiltration of cells. (Haematoxylin and eosin, × 385).

Low dose tolerance or high dose tolerance was induced by the series of the injection of tolerogenic sBSA as described in the Materials and Methods section. One week after the last injection of the tolerogen, mice were immunized by a s.c. injection of

FCA-BSA. A group of mice not receiving sBSA and that receiving neither sBSA nor FCA-BSA served as positive and negative controls, respectively. Three weeks after the immunization, all the mice were challenged with AP-DNP-BSA and 4 days later anti-DNP PFC in the spleen was determined. As shown in Table 5, the number of PFC observed in the tolerized groups was the same as, or even below the level caused by the nonspecific stimulation (Table 1). This indicated that the anti-hapten antibody response was completely abrogated by the tolerance induction to the carrier protein.

The following experiment was performed to investigate the effect of tolerance induction on the development of DTH. Groups of low dose tolerant mice and high dose tolerant mice were immunized with FCA-BSA 1 week after the last injection of the tolerogen. Mice given FCA-BSA alone and those left untreated served as controls. The footpad test was performed 3 weeks after the immunization, and the swelling of footpads at the 3rd and the 48th h after the local challenge was measured. Shortly afterwards, mice were killed and their feet were fixed for microscopic observation. Results shown in Fig. 1 seemed to indicate that both Arthus reactivity and DTH were completely suppressed by the tolerance induction irrespective of the dose of tolerogen. This was especially clear in Arthus reactivity. The difference in the level of DTH between low dose tolerant group and high dose tolerant group was evident by microscopic observation. As shown in Fig. 2, for mice of low dose tolerance, marked infiltration of mononuclear cells was noticed 48 h after the local challenge. In contrast, no histological sign of delayed reaction was observed in mice of high dose tolerance (Fig. 3).

DISCUSSION

Although the helper activity for anti-DNP antibody production in response to DNP-BSA was induced in mice by either i.v. injection of AP-BSA plus ET or s.c. injection of FCA-BSA, only the latter was effective in inducing DTH. This seems to indicate that the helper activity and DTH are mediated by different subsets of T cells. Similar assumptions have been proposed by others studying in different experimental systems; with SRBC in mice (Gordon & Yu, 1973; Tamura & Egashira, 1975), with mastocytoma cells in mice (Dennert & Lennox, 1974; Igarashi, Okada, Kishimoto & Yamamura, 1975), or with salmonella flagellin in rats (Liew & Parish, 1974). Recent studies by Elliott, Haskill & Axelrad (1975) and Elliott & Haskill (1975) showed that cells involved in cell-mediated immunity and those in helper activity specific to SRBC fell into different fractions in velocity sedimentation at unit gravity.

The fact that different cells are involved in helper function and in DTH does not necessarily indicate that they are derived from different precursors. As to the origin of these two subgroups of functional T cells, the following three possibilities have been proposed by several authors (Segal, Cohen & Feldman, 1972; Gordon & Yu, 1973). (1) They are derived from antigen-activated common precursor cells which differentiate along one pathway—the different activities being related to the stage of differentiation. (2) They are derived from common precursor cells which differentiate along separate pathways in response to the different immunogenic information. (3) They are derived from different precursor cells which may be activated by antigenic stimulation and then differentiate independently of each other.

The present study does not completely exclude any one of these three possibilities. The first possibility, however, seems to be supported only feebly, since the helper activity alone was induced by the injection of AP-BSA plus ET without any sign of DTH for 12 weeks (Table 3; see also Katsura *et al.*, 1976b). Immune deviation (Asherson & Stone, 1965) or the deviation-like phenomenon reported previously (Katsura, 1976) appears to conform to the second possibility but not to the third possibility. In contrast, the third possibility is simply the most acceptable for the explanation of the results of the tolerance induction. This point will be discussed after the consideration of the results of tolerance induction.

Much of the work on tolerance induction concerning both DTH and antibody production was done in guinea-pigs. For example, Borel *et al.* (1966) reported that the injection of DNP-bovine gammaglobulin into newborn guinea-pigs caused the abrogation of DTH to DNP in nearly 50 per cent of the animals without affecting the antibody response. More recently, Silver & Benacerraf (1974) have offered an inverse conclusion. Injection of deaggregated human gammaglobulin (HGG) at a low dose into adult guinea-pigs completely

inhibited the development of helper cells which otherwise would have occurred after the challenge with DNP-HGG. However, a higher dose was needed to reduce the development of DTH to the carrier protein. This seems entirely compatible with our results, namely, that the development of DTH was completely abrogated only in high dose tolerant mice whereas the low dose of tolerogen was sufficient for preventing the helper function. Abrogation of helper activity may not be attributable to the generation of suppressor cells by the tolerogen, as we have never succeeded in the detection of suppressor cells specific to BSA (unpublished data). Accordingly, the data here on the tolerance experiments may be interpreted as showing that the precursor of helper cells and that of DTH-effector are different from each other and thus have different susceptibilities to the tolerogen.

If we accept the existence of different precursors which give rise to distinct functional cells, the immune deviation may be explained as the following: the products produced after the 'deviating' immunization, such as antibodies or antigen–antibody complexes, generally suppress induction or manifestation of DTH, as expected in the response of mice to SRBC (Mackaness, Lagrange, Miller & Ishibashi, 1974). This may not, however, be the case in the immune deviation studied by Asherson (1966) or the deviation-like phenomenon in our previous study (Katsura, 1976), since the serum factor(s) affected neither the induction nor the expression of DTH.

Both the differential induction of tolerance and the occurrence of the deviation may be explained, if we assume that two types of antigen-reactive precursor cells are derived from a common progenitor-cell compartment. These progenitor cells are supposed to be antigen-specific but not antigen-sensitive. When a certain immunogenic information stimulates one type of precursor to differentiate and proliferate, the progenitor cell will differentiate to replenish the precursor cell compartment. The other type of precursor cell, which might be over-produced as a necessary consequence of this process, may die within a short period if it does not meet the immunogen.

ACKNOWLEDGMENTS

The authors are most indebted to Dr H. Kawaji (Takeda Chemical Indust. Company Ltd) for the guidance in the histological study and to Ms Yuko Takaoki for her skillful technical assistance and typewriting the manuscript. This work was supported by grant number 911208 from the Ministry of Education, Government of Japan.

REFERENCES

ASHERSON G.L. (1966) Selective and specific inhibition of 24-hour skin reactions in the guinea-pig. II. The mechanism of immune deviation. *Immunology*, **10**, 179.

ASHERSON G.L. & STONE S.H. (1965) Selective and specific inhibition of 24-hour skin reactions in the guinea-pig. I. Immune deviation: description of the phenomenon and the effect of splenectomy. *Immunology*, **9**, 205.

BOREL V., FAUCONNET M. & MIESCHER P.A. (1966) Selective suppression of delayed hypersensitivity by the induction of immunologic tolerance. *J. exp. Med.* **123**, 585.

DAUPHINEE M.J. & NORDIN A.A. (1974) Studies of the immunological capacity of germ-free mouse radiation chimeras. IV. Cell-mediated immunity. *Cell. Immunol.* **14**, 394.

DENNERT G. & LENNOX E.S. (1974) Cooperation and cell-mediated cytotoxicity as functions of two subsets of T cells. *J. Immunol.* **113**, 1553.

ELLIOTT B.E. & HASKILL J.S. (1975) Rosette-forming ability of thymus-derived lymphocytes in humoral and cell-mediated immunity. II. Helper cell activity. *J. exp. Med.* **141**, 600.

ELLIOTT B.E., HASKILL J.S. & AXELRAD M.A. (1975) Rosette-forming ability of thymus-derived lymphocytes in cell-mediated immunity. I. Delayed hypersensitivity and *in vitro* cytotoxicity. *J. exp. Med.* **141**, 584.

GORDON J. & YU H. (1973) Relationship of T cells involved in cell-mediated immunity and antibody synthesis. *Nature: New Biology*, **244**, 21.

IGARASHI T., OKADA M., KISHIMOTO S. & YAMAMURA Y. (1975) The relation between the T cells responsible for cell-mediated cytotoxic killing of Mastocytoma cells and the helper-cell effect. *Immunology*, **28**, 37.

KATSURA Y. (1972) Studies on γM and γG antibody response of mice to bovine serum albumin. II. Induction of tolerance. *Japan. J. Microbiol.* **16**, 269.

KATSURA, Y. (1976) Cell-mediated and humoral immune responses in mice. III. Dynamic balance between delayed-type hypersensitivity and antibody response. *Immunology*, **32**, 227.

KATSURA Y., NAKANO K., KOHARA Y. & UESAKA I. (1976a) Cell-mediated and humoral immune responses in mice. I. Necessary conditions for the detection of delayed-type hypersensitivity. *Int. Arch. Allergy*, (In press.)

KATSURA, Y., INABA K., IZUMI T. & UESAKA I. (1966) Cell-mediated and humoral immune responses in mice. II. Sensitizing conditions for delayed-type hypersensitivity. *Int. Arch. Allergy*, (In press).

LITTLE J.R. & EISEN H.N. (1967) *Methods in Immunology and Immunochemistry* (ed. by C. A. Williams and M. W. Chase), p. 128. Academic Press, New York and London.

LIEW R.Y. & PARISH C.R. (1974) Lack of a correlation

between cell-mediated immunity to the carrier and the carrier-hapten helper effect. *J. exp. Med.* **139,** 779.

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

MURAMATSU S., AMAGAI T. & KATSURA Y. (1975) Tolerance induction in TxXBT and TxXB mice. *Immunology*, **28,** 943.

NORDIN A.A. & FARRAR J.J. (1974) Studies of the immunological capacity of germfree mouse radiation chimeras. III. *In vitro* reconstitution of the T-helper cell deficiency. *Cell Immunol.* **10,** 218.

RITTENBERG M.B. & PRATT K.L. (1969) Antitrinitrophenyl (TNP) plaque assay. Primary response of Balb/c mice to soluble and particulate immunogen. *Proc. soc. exp. Med.*, **132,** 575.

SEGAL S., COHEN I.R. & FELDMAN M. (1972) Thymus-derived lymphocytes: Humoral and cellular reactions distinguished by hydrocortisone. *Science*, **175,** 1126.

SILVER J. & BENACERRAF B. (1974) Dissociation of T cell helper function and delayed hypersensitivity. *J. Immunol.* **113,** 1872.

TAKAOKI M., KAWAGUCHI S., KATSURA Y. & MURAMATSU S. (1976) Tansition in the character of immunological memory in mice after immunization. I. Memory for IGM and IgG antibody responses. *Japan. J. Microbiol.* **20,** 255.

TAMURA S. & EGASHIRA Y. (1975) Cellular and humoral immune responses in mice. II. Effect of intraperitoneal or subcutaneous injection of carrier on anti-hapten antibody and delayed hypersensitivity responses. *Immunology*, **28,** 909.