Immunology 1978 **35** 721

Distinction between suppressors of the delayed-type hypersensitivity and the humoral response to sheep erythrocytes

K. YAMAGUCHI & S. KISHIMOTO *Second Department of Internal Medicine, Kumamoto University Medical School, Kumamoto, Japan* 860

Received 19 *January* 1978; *accepted for publication* 16 *February* 1978

Summary. Suppressors for both delayed-type hypersensitivity (DTH) and the humoral immune response could be simultaneously induced in the spleens of mice by immunization with a high dose of SRBC.

Normal recipient mice of the spleen cells from donors immunized 5 days previously elicited depressed DTH or humoral response when immunized with SRBC. The suppressive activity was found to reside in T not B enriched fraction. Four hundred rad irradiation of the primed spleen cells resulted in complete loss of DTH suppressor activity, but only in some reduction of the suppressor activity for the humoral response. In contrast, hydrocortisone treatment of the donor mice caused no loss of DTH suppressor activity while approximately half of the suppressive activity for anti-SRBC PFC response was lost. Adult thymectomy prevented completely the induction of the DTH suppressor in contrast to little loss of the suppressor activity for the humoral response. DTH suppression was antigen-specific for the induction, but nonspecific for the expression. However the suppression of the humoral response was antigen-specific not only for the induction but also for the expression. In addition, DTH suppressor was capable of suppressing both the induction and expression of DTH while the humoral response was suppressed only in the induction stage by the suppressor.

Correspondence: Dr S. Kishimoto, Second Department of Internal Medicine, Kumamoto University Medical School, 2-1, Honjo 2-chome, Kumamoto City, Kumamoto 860, Japan.

0019-2805/78/1100-0721$20.00

INTRODUCTION

Recently considerable attention has been focused on the existence of heterogeneity in T-cell subpopulations of mice in terms of their functions and surface markers. Among them suppressor T cells have been known to play an important role in the regulation of the immune responses. Their suppressive action has been demonstrated in the cell-mediated immunity of GVH, MLR, cytotoxic allograft response, tumour immunity and DTH (Gershon, Cohen, Hencin & Liebhaber, 1972; Hardin, Chused & Steinberg, 1973; Folch & Waksman, 1974; Simpson & Cantor, 1975; Gorczynski, 1974; Morikawa, Baba, Harada & Mitsuoka, 1977). They are also shown to participate in the regulation of the humoral immune responses. This wide range of responses in which T-dependent regulatory influences are demonstrable implies that suppression is of comparable biological importance to co-operation. In addition, it would seem likely that in these various experimental models, we are studying a number of related suppressor T-cell systems that may differ from one another in a number of respects.

However, it is not yet known whether distinct subclasses of suppressor T cells exist corresponding to the different classes of immune responses.

In this report we are concerned with whether suppressor T cells for DTH to SRBC are distinct from those for the humoral immune response to SRBC and we shall show that suppressor T cells for both DTH and the humoral response can be simultaneously induced in mice immunized with a high dose of SRBC and that suppressor T cells for DTH are different from those for the humoral response in the sensitivities to irradiation and corticosteroid, adult thymectomy effect, and the modes of their actions.

MATERIALS AND METHODS

Mice

Inbred 8 to 12-week-old male C57BL/6J mice were used. Mice were fed a commercial laboratory chow and water *ad libitum*.

Antigens

The same lots of sheep red blood cells (SRBC) or horse red blood cells (HRBC) were used throughout the experiments. Cells in Alsever's solution were purchased by the Chemo-Sero-Therapeutic-Research Institute (Kumamoto, Japan). Cells were washed three times with MEM (Nissui Seiyaku Co., Japan) before use.

Immunization

Mice were immunized i.v. with 10^5 SRBC for DTH or 10^8 SRBC for the humoral response. Assays were done on day 4 for DTH and on day 5 for HA in sera and PFC in spleens.

Test for delayed-type hypersensitivity (DTH)

A modified method of Miller, Mackaness & Lagrange (1973) was employed. DTH was expressed as the increase in footpad thickness 24h after injecting an eliciting dose of 1×10^8 washed SRBC in a volume of 0·02 ml into subcutaneous tissue on the surface of one hind footpad. The dorso-ventral thickness of the hind footpad was measured at 24h with a dial-gauge caliper reading to 0·05mm (Kori Seiki, made in Japan) and expressed in units of 10^{-2}cm.

Assay for antibody forming cells

Direct anti-SRBC antibody forming cells (PFC) were assayed by a haemolytic plaque technique of Cunningham & Szenberg (1968).

Antibody titration

To determine serum antibody titres to SRBC, mice were bled and the sera pooled. The haemagglutinating antibody was titrated using Microplates and the mean titre was expressed in $\log_2$ of the reciprocal of the highest dilution showing haemagglutination. The sensitivity of antibody to 2-mercaptoethanol (2ME) was tested by addition of an equal volume of 0·2M 2ME in saline to the sera at room temperature.

Immune cell transfer

Five days after an intraperitoneal immunization with varying doses of SRBC, mice were killed, the spleens removed and single cell suspensions were prepared. The cells were transferred intraveneously into syngeneic recipients after being thoroughly washed.

Separation of spleen lymphocyte subpopulations

T-enriched fraction. One hundred million spleen cells were incubated at 37° for 60 min on tetron fibre columns (Terumo fibre columns, Jintan-Terumo Tokyo, Japan) equilibrated with RPMI 1640 medium (Nissui Seiyaku) containing 10% foetal calf serum (GIBCO, Grand Island, N.Y.). Among the cells that passed through the column, 94% were positive for the θ antigen and 1% for surface immunoglobulins. *B-enriched fraction.* One volume of spleen cell suspensions at a concentration of 5×10^6/ml were incubated at 37° for 45 min with an equal volume of a 8-fold-diluted rabbit anti-mouse brain-θ associated antigen serum (anti-MBA) and an equal volume of 8-fold-diluted guinea-pig complement. A rabbit anti-MBA was raised by repeated injections of brain of the C3H mice emulsified in Freund's complete adjuvant (Waksal, Pierre, Hostetler & Folk, 1974). The serum was heat-inactivated at 56° for 30 min and absorbed with erythrocytes and livers. After incubation, viable cells were examined for subpopulation. Surface Ig-bearing cells were 94% and θ-positive cells were 2%. The cells were washed twice in MEM before cell transfer.

Corticosteroid treatment

Mice immunized with $1{\cdot}3\times10^{10}$ SRBC were given intraperitoneally 2·5 mg hydrocortisone sodium

succinate (Solu-Cortef, Japan Upjohn limited) 2 days before killing for the cell transfer.

Irradiation

Five days after immunization with 2×10^{10} SRBC, mice were killed and spleen cell suspensions were prepared. The cell suspensions were irradiated *in vitro* with a dose of 200 or 400 rad. The radiation was applied from a ^{60}Co source at a distance of 70 cm at a rate of 50 rad/minute.

Adult thymectomy (ATx)

Mice were thymectomized at the age of 7 weeks by the method of Miller (1960). The same operative procedure was carried out on the same day to prepare sham ATx mice except that the thymic lobes were not removed.

Statistics

Statistical analysis of data was done by Student's *t* test where *P* value <0.05 was considered significant.

RESULTS

The experimental protocol is shown in Fig. 1. In a preliminary study, DTH to SRBC was found to attain the peak on day 4, and no humoral immune response could be induced as detected by haemagglutinating antibody in pooled sera or by PFC in spleen cells when mice were immunized with a dose of 10^5 SRBC. When higher doses than 10^5 SRBC were used as an antigen DTH was substantially depressed, and impossible to detect in mice immunized with a dose of 10^9 SRBC. On the other hand, a dose of 10^8 SRBC revealed to be optimal for the induction of anti-SRBC antibody as detected by either PFC in spleen cells or haemagglutinating antibody in sera, while this dose of SRBC induced only low levels of DTH. Based on these results, mice immunized with 10^5 SRBC were assayed for DTH on day 4 after immunization, whereas those immunized with 10^8 SRBC were assayed for anti-SRBC PFC and haemagglutinating antibody on day 5.

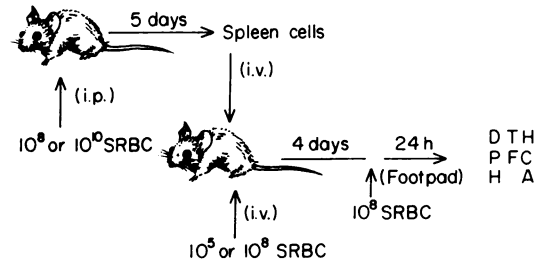

Figure 1. Experimental protocol.

Table 1. Suppression of DTH and antibody response to SRBC in recipient mice of spleen cells from mice immunized with a high dose of SRBC

Cells transferred*	Sensitizing antigen	DTH‡ (Units)	PFC‡ (/10^6SP.C)	HA‡	
				2ME(−)	2ME(+)
Nil†	10^5 SRBC	12·3±0·3			
Immune spleen cells ($1\cdot2\times10^8$ SRBC)§	10^5 SRBC	12·3±0·3			
Immune spleen cells ($2\cdot2\times10^{10}$ SRBC)	10^5 SRBC	6·0±0·2¶			
Nil†	10^8 SRBC		2954±380	5·8±0·3	5·3±0·3
Immune spleen cells ($1\cdot2\times10^8$ SRBC)	10^8 SRBC		2801±197	5·8±0·3	5·5±0·3
Immune spleen cells ($2\cdot2\times10^{10}$ SRBC)	10^8 SRBC		574±175¶	3·0±0·4¶	3·0±0·4¶

* Cells transferred; $4\cdot5\times10^7$ spleen cells.
† No cells transferred; control.
‡ Mean values ± S.E.M. from four to five mice per group.
§ Immunizing dose.
¶ $0.001<P<0.01$

Suppressive activity in the spleens of mice immunized with a high dose of SRBC

Forty five million spleen cells obtained from mice which had been immunized with $1{\cdot}2\times10^{8}$ or $2{\cdot}2\times10^{10}$ SRBC 5 days previously were transferred i.v. into normal syngeneic recipients. Immediately after cell transfers, the recipients were immunized with 10^{5} SRBC for DTH or 10^{8} SRBC for the humoral response, challenged with 10^{8} SRBC for DTH 4 days later, and then assayed for DTH, PFC and HA 24 h later. Table 1 shows that spleen cells from mice immunized with $2{\cdot}2\times10^{10}$ SRBC 5 days previously could suppress both DTH and the antibody response to SRBC when transferred into normal syngeneic recipients. However, no suppression was observed in the recipient mice of spleen cells from mice immunized with $1{\cdot}2\times10^{8}$ SRBC, when they were immunized with SRBC.

To clarify the cell dose–effect relationship, graded numbers of the spleen cells from mice immunized with $1{\cdot}3\times10^{10}$ SRBC 5 days previously were transferred into normal syngeneic recipients. In the recipients of 7×10^{7} or 4×10^{7} immune spleen cells, both DTH and humoral response to SRBC were significantly depressed while the transfer of 2×10^{7} or 1×10^{7} immune spleen cells did not suppress them (Fig. 2).

T cell dependency of suppressive activity

To analyse the cellular basis for this suppression, spleen cells from mice immunized with $1{\cdot}3\times10^{10}$ SRBC were separated into T-and B-enriched cell fractions. Representative results are shown in Tables 2 and 3. The adoptive transfer of T-enriched cell fraction which had been obtained by passing the immune spleen cells through a fibre column showed same degree of suppression as that caused by unfractionated spleen cells whereas B-enriched cell fraction which had been obtained by incubating the immune spleen cells in the anti-MBA serum and complement caused no suppression in the adoptively transferred recipients. These results indicate that T cells, not B cells were responsible for the suppression of DTH and the antibody response.

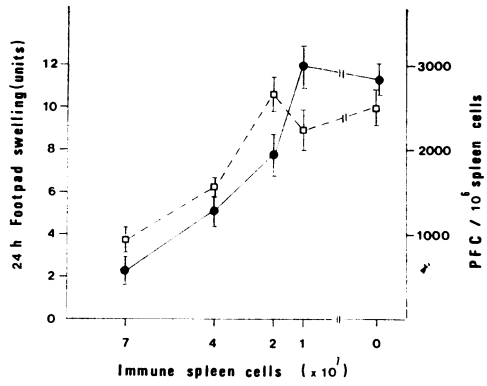

Figure 2. Effect of transferring graded numbers of immune spleen cells on DTH (●) and PFC (□) measured on day 5. Means of 5±SEM.

Radiosensitivity of suppressor cells

To further elucidate whether the suppressor T cells for DTH are distinct from those for the antibody response, the sensitivities of the suppressor T cells to irradiation and corticosteroid were compared. Just before cell transfer, immune spleen cells suspensions from donors immunized with $1{\cdot}5\times10^{10}$ SRBC 5 days previously were irradiated with 0, 200, or 400 rad. The exposure of them to 400 or 200 rad irradiation resulted in complete or partial loss of DTH suppression respectively. In contrast, immune spleen cells irradiated with 400 rad were still capable of suppressing the anti-SRBC response (Table 4).

Corticosteroid sensitivity of suppressor cells

Mice immunized with 2×10^{10} SRBC 3 days previously were injected i.p. with 2·5mg hydrocortisone. Then 2 days later they were killed to make spleen cell suspensions for cell transfer. In recipient mice of hydrocortisone-treated immune spleen cells, DTH was suppressed as markedly as in those of non-treated immune spleen cells when recipient mice were immunized with SRBC (Table 5). In contrast to DTH, the humoral immune response as detected by PFC in spleens and haemagglutinating antibody in sera was not suppressed in mice receiving hydrocortisone-treated immune spleen cells, followed by immunization with SRBC. These results suggest that the suppressor cells for DTH were more hydrocortisone-resistant than those for the humoral immune response.

Effect of adult thymectomy on suppressor cells

Adult mice which had been thymectomized or sham-operated 30 days previously were immunized with $1{\cdot}3\times10^{10}$ SRBC. Five days later the spleen cell suspensions were prepared from these mice for

Table 2. T-cell dependence of DTH suppressor

Cells transferred*	Sensitizing antigen	DTH (Units)
Nil	10^5 SRBC	8·2±0·9
Immune spleen cells ($5·5\times10^7$)	10^5 SRBC	4·5±0·3§
Immune spleen T cells† ($5·0\times10^7$)	10^5 SRBC	3·4±0·7§
Immune spleen B cells‡ ($5·0\times10^7$)	10^5 SRBC	7·8±0·4

* Spleen cells from donors immunized with $1·3\times10^{10}$ SRBC 5 days previously were transferred.
† obtained by passage of a tetron fibre column.
‡ obtained by anti-MBA and complement.
§ $P<0·05$.

Table 3. T-cell dependence of suppressor for antibody response

Cells transferred*	Sensitizing antigen	PFC ($/10^6$SP.C)	HA	
			2ME(−)	2ME(+)
Nil	10^8 SRBC	2432±193	5·0±0·1	4·3±0·2
Immune spleen cells ($4·3\times10^7$)	10^8 SRBC	1168±238§	4·3±0·2¶	4·0±0·4
Immune spleen T cells† ($1·0\times10^7$)	10^8 SRBC	1181±154§	4·3±0·2¶	3·8±0·2
Immune spleen B cells‡ ($4·0\times10^7$)	10^8 SRBC	2717±280	5·0±0·5	5·0±0·4

* Spleen cells from donors immunized with $1·3\times10^{10}$ SRBC 5 days previously were transferred.
†‡ same as legends described in Table 2.
§ $0·001<P<0·01$
¶ $P<0·05$

Table 4. Radiation sensitivity of suppressor cells

Cells transferred* Irradiation dose (rad)	Sensitizing antigen	DTH (Units)	PFC ($/10^6$SP.C)	HA	
				2ME(−)	2ME(+)
Nil	10^5 SRBC	9·7±0·8			
0	10^5 SRBC	3·0±0·7§			
200	10^5 SRBC	7·0±0·2†			
400	10^5 SRBC	10·0±0·4			
Nil	10^8 SRBC		3324±478	5·5±0·5	5·3±0·2
0	10^8 SRBC		1115±174†	3·3±0·3§	2·8±0·2§
200	10^8 SRBC		1311±160†	3·5±0·2§	3·3±0·2§
400	10^8 SRBC		1869±310†	4·0±0·3	3·0±0·3‡

* Spleen cells from donors immunized with $1·5\times10^{10}$ SRBC 5 days previously were transferred.
Cells transferred; $4·6\times10^7$ spleen cells.
† $0·02<P<0·05$
‡ $0·01<P<0·02$
§ $0·001<P<0·01$

Table 5. Corticosteroid sensitivity of suppressor cells

Cells transferred*	Sensitizing antigen	DTH (Units)	PFC (/10^6SP.C)	HA	
				2ME(−)	2ME(+)
1. Nil	10^5 SRBC	10·0±0·5			
Immune spleen cells	10^5 SRBC	7·0±0·7†			
C.S. treated immune spleen cells	10^5 SRBC	5·3±1·1†			
2. Nil	10^5 SRBC	12·3±0·4			
Immune spleen cells	10^5 SRBC	5·0±0·7‡			
C.S. treated immune spleen cells	10^5 SRBC	4·5±0·7‡			
3. Nil	10^8 SRBC		1981±162	6·3±0·5	5·8±0·5
Immune spleen cells	10^8 SRBC		1145±38‡	4·3±0·5†	3·8±0·7†
C.S. treated immune spleen cells	10^8 SRBC		1548±220	5·5±0·5	4·5±0·5

* Cells transferred; $4·6\times10^7$ spleen cells obtained from mice immunized with 2×10^{10} SRBC and 3 days later given no or 2·5 mg hydrocortisone.
† $0·01 < P < 0·02$
‡ $P < 0·001$

transfer into normal recipient mice. Mice receiving the immune spleen cells from thymectomized mice could elicit a DTH response comparable to that elicited by control mice not receiving spleen cells, while they produced suppressed anti-SRBC response. On the other hand, the immune spleen cells from sham-operated donors could suppress both DTH and the anti-SRBC response when transferred to normal recipients. As shown in Table 6, adult thymectomy completely abolished the induction of DTH suppressor activity whereas it caused little effect on the induction of suppressor activity for the humoral immune response.

Specificity of suppressor cells

Spleen cells from donor mice immunized with SRBC or HRBC 5 days previously were transferred into normal recipient mice which were then immunized

Table 6. Effect of ATx on suppressor cells

Cells transferred†	Sensitizing antigen	DTH (Units)	PFC (/10^6SP.C)	HA	
				2ME(−)	2ME(+)
Nil	10^5 SRBC	9·5±1·0			
Immune spleen cells (sham-op)	10^5 SRBC	5·0±0·8‡			
ATx immune spleen cells*	10^5 SRBC	12·0±1·0			
Nil	10^8 SRBC		4195±330	5·5±0·3	5·3±0·4
Immune spleen cells (sham-op)	10^8 SRBC		864±117‡§	3·8±0·2‡	3·3±0.2‡§
ATx immune spleen cells	10^8 SRBC		1477±138‡**	4·0±0·1‡	4·0±0·1‡**

* Adult thymectomy performed 30 days previously.
† Spleen cells from donors immunized with $1·3\times10^{10}$ SRBC 5 days previously were transferred. Cells transferred; $4·2\times10^7$ spleen cells.
‡ $P < 0·001$
§ vs
** $0·01 < P < 0·02$

with 10^5 SRBC, 10^5 HRBC or $0{\cdot}5\times10^5$ SRBC and $0{\cdot}5\times10^5$ HRBC, and challenged with SRBC for footpad reaction on day 4. As shown in Table 7, spleen cells from donor mice immunized with SRBC suppressed DTH whereas those with HRBC did not suppress at all. However, it is interesting that the recipients of spleen cells from donor mice immunized with HRBC showed suppressed DTH to SRBC when simultaneously immunized with HRBC and SRBC. This indicates that the suppression is specific for the induction, but non-specific for the expression. Similarly, recipient mice of HRBC-immune spleen cells did not suppress anti-SRBC PFC response. In contrast to DTH suppressor T cells the suppression of the humoral response was considered specific for both induction and expression since the transfer of HRBC-immune spleen cells failed to suppress anti-SRBC PFC response even when recipient mice were immunized with SRBC and HRBC (Table 8).

Stage of immune response where suppressor cells exert their effect

The immune spleen cells from donors immunized with SRBC 5 days previously could depress DTH when transferred to normal recipient mice just before or 4 days after immunizing the recipients (Table 7). By contrast, in the recipient mice anti-SRBC response was substantially suppressed on the transfer of the immune spleen cells only before immunization, but not 4 days after immunization (Table 8). These results indicate that the suppressor for DTH can exert its activity in both induction and effector stages whereas that for the humoral response is exerted only in the induction stage.

DISCUSSION

The data described above demonstrate that suppressors for both DTH and the humoral immune response can be simultaneously induced in the spleen cells of mice by immunization with a high dose of SRBC. This suppression was manifested by transferring the spleen cells from donors immunized 5 days previously into normal syngeneic recipients which elicited depressed DTH or humoral response as compared to the responses of control mice without receiving transfer of the immune spleen cells when immunized with SRBC. These results seem to be similar, but not identical to recent reports of Ramshaw, Bretscher & Parish (1976) which show

Table 7. Specificity of DTH suppressor and stage of immune response where suppressor cells exert their effects

Cells transferred*	Sensitizing antigen	DTH (Units)
Nil	10^5 SRBC	10·0±1·0
Nil	10^5 HRBC	7·8±0·6§
Immune spleen cells ($1{\cdot}3\times10^{10}$ SRBC)†	10^5 SRBC	6·6±0·8*
Immune spleen cells ($1{\cdot}3\times10^{10}$ HRBC)	10^5 HRBC	2·8±0·4§††
Immune spleen cells ($1{\cdot}3\times10^{10}$ HRBC)	10^5 SRBC	10·5±0·8
Immune spleen cells ($1{\cdot}3\times10^{10}$ HRBC)	$0{\cdot}5\times10^5$ SRBC + $0{\cdot}5\times10^5$ HRBC	5·2±1·0**
Immune spleen cells‡ ($1{\cdot}3\times10^{10}$ SRBC)	10^5 SRBC	4·6±0·8*

* Cells transferred; $4{\cdot}2\times10^7$ spleen cells.
† Immunizing dose.
‡ Immune spleen cells transferred to recipient mice that had been immunized 4 days previously.
§ HRBC were used in the assay.
¶ $P<0{\cdot}001$ **$0{\cdot}01<P<0{\cdot}02$
††$0{\cdot}001<P<0{\cdot}01$

Table 8. Specificity of the humoral suppressor cells and stage of immune response where suppressor cells exert their effects

Cells transferred*	Sensitizing antigen	PFC (/10^6SP.C)	HA	
			2ME(−)	2ME(+)
Nil	10^8 SRBC	1934±185	5·0±0	4·8±0·2
Nil	10^8 HRBC	771± 98§	5·5±0·2§	4·8±0·3§
Immune spleen cells (1.3×10^{10} SRBC) †	10^8 SRBC	517±52¶	3·2±0·2¶	1·6±0·7¶
Immune spleen cells ($1\cdot3\times10^{10}$ HRBC)	10^8 HRBC	152±14§¶	3·6±0·3§¶	2·8±0·4§¶
Immune spleen cells ($1\cdot3\times10^{10}$ HRBC)	10^8 SRBC	1685±275	5·2±0·4	5·0±0·3
Immune spleen cells ($1\cdot3\times10^{10}$ HRBC)	$0\cdot5\times10^8$ SRBC + $0\cdot5\times10^8$ HRBC	1455±220	5·3±0·3	5·2±0·4
Immune spleen cells‡ ($1\cdot3\times10^{10}$ SRBC)	10^8 SRBC	1526±338	4·5±0·3	4·3±0·3

* Cells transferred; $4\cdot2\times10^7$ spleen cells.
† Immunizing dose.
‡ Immune spleen cells transferred to recipient mice that had been immunized with SRBC 4 days previously.
§ HRBC were used in the assay.
¶ $P<0\cdot001$

that the Ig^- spleen cells from mice immunized with 10^9 HRBC could suppress DTH to HRBC when transferred to cyclophosphamide-treated recipient mice, but there was no evidence for the emergence of suppressors for the humoral response to HRBC in donor mice. Furthermore, in their following experiment they could induce specific antibody unresponsiveness in adult CBA/H mice by the injection of cyclophosphamide 24 h after immunization with a large dose of HRBC (Ramshaw, Bretscher & Parish, 1977a). However, although the mice were unresponsive to HRBC at the humoral level via active T-cell suppression, they simultaneously expressed high levels of DTH to HRBC. In contrast, we could induce suppressors for both DTH and the humoral response in the same donor mice immunized with a high dose of SRBC. This, therefore, has led us to make comparisons between suppressor for DTH and one for the humoral response. The discrepancy between our data and their data may be accounted for by the administration of cyclophosphamide to the donor mice after immunization with a large dose of HRBC in Ramshaw's experiments.

Cells responsible for the suppression are T cells, not B cells or macrophages, because the T-cell-enriched fraction of the immune spleen cells was capable of suppressing both immune responses whereas the B cell-enriched fraction failed to cause the suppression when transferred to naive recipients. In this context, Basten, Miller & Johnson (1975) have shown that T-dependent suppression was not mediated by a purified population of tolerant T cells, but required the presence of an adherent cell, probably a tolerant macrophage, for the suppression of the humoral response to DNP-HGG. Similar findings were also reported in the studies of Zembala & Asherson (1973) on inhibition of contact sensitivity. Our data that T cells are essential for the suppression do not necessarily mean that T cells are the only type of cells responsible for the induction of the suppression. This is because it could be possible for T cells to cause the suppression in collaboration with macrophages contaminating the T-cell-enriched fraction. However, this possibility seems unlikely to us. No evidence was obtained for the contribution of B cells or antibody to the suppression which has been claimed by others to participate in the suppression (Katz, Parker & Turk, 1974; Uhr & Baumann, 1961; Neta & Salvin, 1974; Zembala, Asherson, Noworolski & Mayhew, 1976a).

Basten *et al.* (1975) have shown that irradiation completely abrogated the suppression of anti-DNP response when cells from HGG-tolerant mice were exposed to 1000 rad irradiation from ^{60}Co source and assayed in the standard adoptive transfer system.

A similar result was also obtained in the studies of Nachtigal, Zan-Bar & Feldman (1975) on the suppressor T cells in BSA-tolerant mice which inhibited partially the suppression when given 600 rad. In addition, direct *in vitro* irradiation of unprimed spleen cells with 200 or 400 rad has been known to cause partial abrogation of the suppression induced by Con-A. However, these are in contrast to recent findings of Doyle, Parks & Weigle (1976) that the inhibitory activity of the suppressor cells was resistant to 900 rad irradiation regardless of whether the HGG tolerant spleen cells were irradiated before or after adoptive transfer. The radiosensitivity of suppressor T cells for DTH was also examined in the study of Zembala & Asherson (1976) who showed that the suppressor activity was completely lost by 700 rad of whole body irradiation given to the donors 2 days before cell transfer. In our results, both suppressors for DTH and for the humoral response were relatively radiosensitive, although a distinction in the radiosensitivity was observed between both suppressors. The exposure to 200 rad irradiation resulted in 60% loss of the suppressor activity for DTH but in only 11% loss for the humoral response. Four hundred rad irradiation could abolish full activity of the suppressor for DTH, but only 34% of the suppressive activity for the humoral response.

Hydrocortisone injection to mice immunized with a high dose of SRBC 2 days before cell transfer caused approximately 50% loss of the suppressor activity for anti-SRBC PFC response, but no loss for DTH at all. This seems to be in accord with a study of Ha, Waksman & Treffers (1974) on rats. Simultaneous administration of hydrocortisone with a large dose of BGG to rats abolished the suppressive activity for the production of precipitin to BGG, but still retained some suppressive activity for DTH to BGG when thymocytes from donor rats were adoptively transferred to syngeneic recipients that were subsequently immunized with BGG. These results contrast with the data of Nachtigal *et al.* (1975) who found that lymphoid cells of tolerant mice were found equally suppressive before and after cortisone treatment of the tolerant donors when lymphoid cells of tolerant cortisone-treated mice were tested for a suppressive effect in adoptive transfers. In addition, they have assumed that cortisone-resistant mature suppressor T cells arise by differentiation from immature, cortisone-sensitive precursors, in response to a signal transmitted by the antigen.

The presence of intact thymus has been known to be intimately related to the induction of suppressor T cells and there is a direct evidence that ATx interferes with the suppressor T cells which depress the antibody response to HSA in mice (Nachtigal *et al.*, 1975) and the termination of the specific IgE response by suppressor T cells in rats (Okumura & Tada, 1971). Here again, we have observed a differential effect of ATx on the induction of the suppressors. ATx prevented completely the induction of the suppressor for DTH in contrast to only 11% loss of the suppressor activity for the anti-SRBC PFC response. Asherson, Zembala, Mayhew & Goldstein (1976) have indicated that ATx prevented the appearance of suppressor T cells following the injection of picryl sulphonic acid into donor mice. This effect of ATx was reversed by the thymus graft or thymic extracts. Thus, they have argued that the suppressor T cells for DTH are dependent on the presence of the thymus because they require a thymus hormone, and not primarily because they belong to a short-lived population which is rapidly renewed by cells coming from the thymus. Whatever the mechanism(s) for the prevention of the suppressor appearance by ATx, our data clearly indicate that the suppressor for DTH is more dependent on the presence of the thymus than that for humoral response.

The induction of DTH suppression was antigen-specific a sense that HRBC-immune spleen cells were capable of suppressing DTH to HRBC but not SRBC in the recipient mice. This, however, is in conflict with a previous study of Ramshaw *et al.* (1976) that HRBC-immune spleen cells suppressed, to some extent, DTH to SRBC which were cross-reactive at the helper T-cell level when transferred into syngeneic normal recipients. The reason for this discrepancy is unknown at present. The expression of DTH suppression, however, was non-specific since the transfer of HRBC-immune spleen cells could completely suppress DTH to SRBC induced by immunization with a mixture of SRBC and HRBC in recipient mice. This may be explained by assuming that a soluble factor released by HRBC-immune spleen cells on stimulation with HRBC may nonspecifically suppress the induction of DTH to SRBC. In this context, it is known that a soluble factor elaborated from the cultures of lymph node cells of picryl sulphonic acid-treated mice can suppress the response to picryl chloride and oxazolone when recipient mice are challenged simul-

taneously with picryl chloride and oxazolone (Zembala, Asherson, Mayhew & Krejci, 1975). In contrast, the suppression of the humoral response was antigen-specific not only for the induction but also for the expression, since the transfer of HRBC-immune spleen cells failed to suppress anti-SRBC response induced by immunization with a mixture of HRBC and SRBC in recipient mice. This result seems relevant to data of Tada, Taniguchi & Takamori (1975) that a soluble suppressive factor obtained from thymus of donor mice immunized with a carrier protein is antigen-specific. DTH suppressor was capable of suppressing both the induction and expression of DTH while the humoral response was suppressed only in the induction stage by the suppressor. Numbers of anti-SRBC were not diminished when suppressor was adoptively transferred to recipient mice which had been immunized 4 days previously. This may suggest that the suppressor does not exert its effect on actively antibody-forming cells.

Recent elegant work on T-cell subclasses has shown that the suppressors involved in the cell-mediated immunity and the humoral response express the Ly-2, 3^+ phenotype and that Ly-1^+, 2^+, 3^+ cells may be required for optimal generation of the Ly-2, 3^+ suppressor cells after stimulation by modified-self (Vadas, Miller, Mckenzie, Chism, Shen, Boyse, Gamble & Whitelaw, 1976; Cantor & Boyse, 1975a,b). The properties previously attributed to T_1 cells are shared by at least a portion of the cells in the Ly-1^+, 2^+, 3^+ T subclass which is usually reduced by 50% in the spleen 4 weeks after adult thymectomy (Cantor & Boyse, 1975a). Taken together with these data one may postulate that the DTH suppressor is generated from the precursor of the Ly-1^+, 2^+, 3^+ T subclass or in the presence of the Ly-1^+, 2^+, 3^+ T subclass and rapidly proliferating cell while the generation of suppressor for the humoral response does not depend upon the presence of the Ly-1^+, 2^+, 3^+ T subclass and the suppressor for the humoral response is slowing proliferating cell. The sensitivities to irradiation and hydrocortisone also suggests that the DTH suppressor is distinct from the one for the humoral response. During the preparation of this manuscript, Ramshaw, McKenzie, Bretscher & Parish (1977b) have presented evidence indicating that the suppressor T cell of DTH is Ly-1^+, Ly-2^- and Ia^- whereas the suppressor T cell of humoral immunity is Ly-1^-, Ly-2^+ and Ia^+. This seems to be in support of our results. Whether these cells are derived from separate precursors or represent different maturation stages of a common precursor has not been determined.

REFERENCES

Asherson G.L., Zembala M., Mayhew B. & Goldstein A. (1976) Adult thymectomy prevention of the appearance of suppressor T cells which depress contact sensitivity to picryl chloride and reversal of adult thymectomy effect by thymus extract. *Eur. J. Immunol.* **6**, 699.

Basten A., Miller J.F.A.P. & Johnson P. (1975) T cell-dependent suppression of an anti-hapten antibody response. *Transplant. Rev.* **26**, 130.

Cantor H. & Boyse E.A. (1975a) Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. *J. exp. Med.* **141**, 1376.

Cantor H. & Boyse E.A. (1975b) Functional subclasses of T Lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly^+ cells in the generation of killer activity. *J. exp. Med.* **141**, 1390.

Cunningham A. & Szenberg A. (1968) Further improvements in the plaque technique for detecting single antibody forming cells. *Immunology*, **14**, 599.

Doyle M.V., Parks D.E. & Weigle W.O. (1976) Specific, transient suppression of the immune response by HGG tolerant spleen cells. II. Effector cells and target cells. *J. Immunol.* **117**, 1152.

Folch H. & Waksman B.H. (1974) The splenic suppressor cell. II. Suppression of the mixed lymphocyte reaction by thymus-dependent adherent cells. *J. Immunol.* **113**, 140.

Gershon R.K., Cohen P., Hencin R. & Liebhaber S.A. (1972) Suppressor T cells. *J. Immunol.* **108**, 586.

Gorczynski R.M. (1974) Immunity to murine sarcoma virus-induced tumours. II. Suppression of T cell-mediated immunity by cells from progressor animals. *J. Immunol.* **112**, 1826.

Ha T.-Y., Waksman B.H. & Treffers H.P. (1974) The thymic suppressor cell. I. Separation of subpopulations with suppressor activity. *J. exp. Med.* **139**, 13.

Hardin J.A., Chused T.M. & Steinberg A.D. (1973) Suppressor cells in the graft vs host reaction. *J. Immunol.* **111**, 650.

Katz S.I., Parker D. & Turk J.L. (1974) B-cell suppression of delayed hypersensitivity reactions. *Nature*, **251**, 550.

Miller J.F.A.P. (1960) Studies on mouse leukaemia. The role of the thymus in leukaemogenesis by cell-free leukaemic filtrates. *Brit. J. Cancer*, **14**, 93.

Miller T.E., Mackaness G.B. & Lagrange P.H. (1973) Immunopotentiation with BGG. II. Modulation of the response to sheep red blood cells. *J. Nat. Cancer Inst.* **51** 1669.

Morikawa S., Baba M., Harada T. & Mitsuoka A. (1977) Studies on delayed hypersensitivity in mice. III. Evidence for suppressive regulatory T_1-cell population in delayed hypersensitivity. *J. exp. Med.* **145**, 237.

NACHTIGAL D., ZAN-BAR I. & FELDMAN M. (1975) The role of specific suppressor T cells in immune tolerance. *Transplant. Rev.* **26**, 87.

NETA R. & SALVIN S.B. (1974) Specific suppression of delayed hypersensitivity: The possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. *J. Immunol.* **113**, 1716.

OKUMURA K. & TADA T. (1971) Regulation of homocytotropic antibody formation in the rats. III. Effect of thymectomy and splenectomy. *J. Immunol.* **106**, 1019.

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

RAMSHAW I.A., BRETSCHER P.A. & PARISH C.R. (1977a) Regulation of the immune response. II. Repressor T cells in cyclophosphamide-induced tolerant mice. *Eur. J. Immunol.* **7**, 180.

RAMSHAW I.A., MCKENZIE I.F.C., BRETSCHER P.A. & PARISH C.R. (1977b) Discrimination of suppressor T cells of humoral and cell-mediated immunity by anti-Ly and anti-Ia sera. *Cell. Immunol.* **31**, 364.

SIMPSON E. & CANTOR H. (1975) Regulation of the immune response by subclasses of T lymphocytes. II. The effect of adult thymectomy upon humoral and cellular response in mice. *Eur. J. Immunol.* **5**, 337.

TADA T., TANIGUCHI M. & TAKEMORI T. (1975) Properties of primed suppressor T cells and their products. *Transplant. Rev.* **26**, 106.

UHR J.W. & BAUMANN J.B. (1961) Antibody formation. I. The suppression of antibody formation by passively administered antibody. *J. exp. Med.* **113**, 935.

VADAS M.A., MILLER J.F.A.P., MCKENZIE I.F.C. CHISM S.E., SHEN F.W., BOYSE E.A., GAMBLE J.R. & WHITELAW A.M. (1976) Ly and Ia antigen phenotypes of T cells involved in delayed-type hypersensitivity and in suppression. *J. exp. Med.* **144**, 10.

WAKSAL S.D., PIERRE R.L.S., HOSTETLER J.R. & FOLK R.M. (1974) Brain-associated-theta antiserum. Differential effect on lymphocyte subpopulations. *Cell. Immunol.* **12**, 66.

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

ZEMBALA M., ASHERSON G.L., MAYHEW B. & KREJCI J. (1975) *In vitro* absorption and molecular weight of specific T-cell suppressor factor. *Nature*, **253**, 72.

ZEMBALA M., ASHERSON G.L., NOWOROLSKI J. & MAYHEW B. (1976a) Contact sensitivity to picryl chloride: The occurrence of B suppressor cells in the lymph nodes and spleen of immunized mice. *Cell. Immunol.* **25**, 266.

ZEMBALA M. & ASHERSON G.L. (1976) The effect of cyclophosphamide and irradiation on cells which suppress contact sensitivity in the mouse. *Clin. exp. Immunol.* **23**, 554.