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Suppressive Effect of Graft versus Host Reactions on the Immune Response to Heterologous Red Cells

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Summary. Graft versus host (GVH) reactions induced by the inoculation of parental spleen cells into adult untreated F_1 recipients caused a marked suppression of the cellular and humoral immune response to sheep red cells (SRBC) and *Escherichia coli* lipopolysaccharide, provided the GVH reaction was induced 7 days before immunization with SRBC. Adoptive transfer of parental or F_1 spleen cells mixed with SRBC into irradiated F_1 recipients, which had been subjected to a GVH reaction for 7 days, resulted in marked suppression of cellular antibody synthesis to both antigens.

When the GVH reaction was induced by parental spleen cells from donors immunized to SRBC and the immune response to SRBC tested after 7 days, a marked suppression of cellular antibody synthesis occurred when the parental cells were of *H*-2^b genotype, but not if they were of *H*-2^a or *H*-2^k genotypes.

The number of antigen-sensitive cells of parental genotype sensitive to SRBC in animals being subjected to a GVH reaction for 7 days was unaffected when the donors were of *H*-2^a or *H*-2^k genotypes, but decreased when the donors were *H*-2^b. The number of antigen-sensitive cells of F_1 genotype was only slightly decreased by a GVH reaction.

It is suggested that the suppressive effect of a GVH reaction on antibody synthesis to other antigens represents an example of antigenic competition. This phenomenon would be caused by suppressed proliferation of immunocompetent cells of bone-marrow origin and not by competition for pluripotent antigen-sensitive cells. This suppression would be mediated by antigen stimulated thymus-derived lymphocytes.

INTRODUCTION

The graft versus host (GVH) reaction caused by the inoculation of immunocompetent lymphoid cells of parental origin into untreated adult F_1 hybrid recipients was found to suppress the ability of the recipients to produce antibodies to sheep red cells (SRBC) (Lawrence and Simonsen, 1967). It was demonstrated that the GVH reaction should occur from 7 to 10 days before immunosuppression to SRBC was expressed (Lawrence and Simonsen, 1967). These findings have been interpreted to depend on initially pluripotent immunocompetent cells becoming restricted in their specificity as a consequence of the contact with antigens of the strong *H*-2 system. Thus, the phenomenon of antigenic competition was used as an argument for the existence of pluripotent lymphocytes.

It is well known that parental lymphoid cells exhibit an anomalous behaviour after transplantation into F_1 recipients. Thus, spleen, bone marrow and antibody producing cells show deficient multiplication in F_1 hybrids as compared to syngeneic recipients.

This effect has been attributed to various phenomena, such as death of the cells as a consequence of the GVH reaction (Boyse, 1959), the existence of recessively determined isoantigens (Celada and Welshons, 1962), the allogeneic inhibition phenomenon (Hellström and Möller, 1965) or growth inhibition due to a mismatch with regard to the $H\text{-}2^b$ histocompatibility allele without further specification of the mechanism (Snell and Stevens, 1961; Cudkowicz and Stimpfling, 1964). However, the deficient growth of parental cells in F_1 hybrids appears to be a general phenomenon, which is observed with a variety of proliferating cells, such as neoplasms of sarcoma, carcinoma and lymphoma origin (Hellström and Möller, 1965).

It was previously demonstrated that antigenic competition between sheep and horse red cells only occurred when the injections of the two antigens were spaced in time (Radovich and Talmage, 1967; Möller and Sjöberg, 1970). The phenomenon was not due to competition for common antigen-sensitive cells, because the number of cells reacting with one antigen was unaffected by previous treatment of the host with another antigen (Möller and Sjöberg, 1970). However, animals subjected to previous treatment with one antigen were found to be unable to support the immune response of adoptively transferred immunocompetent lymphoid cells even when they were irradiated, suggesting that antigen injections altered the recipients in such a way that they could not support proliferation of transferred immunocompetent cells (Möller and Sjöberg, 1970). Therefore, it was of interest to investigate whether the GVH reaction, representing an uninterrupted antigenic stimulation on the part of the parental cells, would cause analogous changes. Thus, the effect of a GVH reaction on the immune response to SRBC was investigated with particular emphasis on its effect on the number of antigen-sensitive cells sensitive to SRBC and on its ability to alter the capacity of the recipients to support the immune response of adoptively transferred immunocompetent cells of different genotypes. Furthermore, the kinetics of the immunosuppressive effect of a GVH reaction was studied. In general, the results were found to be analogous to those previously reported with sheep and horse red cells (Möller and Sjöberg 1970).

MATERIALS AND METHODS

Mice of the inbred strains A, A.CA, CBA, C57BL, C57L, B10.5M (5M) and F_1 hybrids between them were used for the experiments.

Antigens and immunization. Sheep red blood cells (SRBC) were employed in all experiments. In addition, *E. coli* 055:B5 heat-killed bacteria were used. For routine tests 4×10^8 SRBC were injected intravenously. Other doses and the time of injection will be specified in the text.

Cell suspensions were prepared by pressing spleens through a 60-mesh stainless steel screen into balanced salt solution. Cell clumps were removed by filtration through gauze. The number of trypan-blue unstained cells were counted in a haematocytometer as described before (Möller, 1968).

Detection of antibody-producing cells. These cells were detected by the agar-plaque technique of Jerne and Nordin (1963) for direct plaque-forming cells (PFC) and by the indirect modification described by Dresser and Wortis (1965) for detection of indirect PFC. In the latter test anti-mouse γ-globulin antiserum was raised by immunizing rabbits with complexes of *Salmonella adelaide* bacteria and specific mouse antibodies as described before (Möller, 1968).

In addition, cellular antibody synthesis to *E. coli* endotoxin was studied as described before (Möller, 1965). In short, polysaccharide used for coating the sheep red cells was obtained from *E. coli* 055:B5 bacteria and was extracted as described before (Möller, 1965). A solution containing 1 mg polysaccharide per ml in saline was boiled for 1 hour immediately before use and 1·0 ml was added to 0·1 ml packed and washed sheep red cells. The cells were incubated for 30 minutes at 37°, washed three times in saline and diluted to a concentration of 4×10^8 per ml. These cells were used in the agar plaque assay.

X-irradiation. Mice were irradiated with 350 r or 600 r at a rate of 425 r/min. X-rays were generated in a Siemens X-ray machine at 185 kV and 15 mA and were filtered by 0·5 mm Cu.

Transfer experiments. To study antigen-sensitive cells, a transfer system was used. Spleen cells from normal or immune animals were made up to 50×10^6 cells/ml and mixed with SRBC 0·25 ml of this mixture was given intravenously to 350 r irradiated syngeneic or F_1 recipients, each of them receiving 10^7 spleen cells mixed with 4×10^8 SRBC. 7 days later the number of 19S and 7S PFC was determined in the spleens of the recipients. The cell dose used for transfer is such that the number of PFC appearing in the recipient is proportional to the number of antigen-sensitive cells in the inoculum (Kennedy, Siminovitch, Till, and McCulloch, 1966).

RESULTS

KINETICS OF THE IMMUNOSUPPRESSIVE EFFECTS

It was first demonstrated by Lawrence and Simonsen (1967) that a graft versus host reaction induced by the inoculation of parental spleen cells into adult F_1 hybrids caused a pronounced suppression of the immune response to SRBC, provided that the GVH reaction preceded immunization with SRBC by 7–10 days. Analogous results were later obtained when the immunosuppressive effect of a GVH reaction was studied with regard to homograft reactions against allogeneic skin grafts (Lapp and Möller, 1969). These findings were confirmed in the present study (Table 1). Thus, there was no suppressive effect of a GVH reaction on cellular antibody synthesis to SRBC when parental spleen cells were inoculated into adult F_1 hybrids at the same time as they received SRBC. However, when the SRBC were inoculated 7 or 8 days after the initiation of the GVH reaction there was a marked suppression of the number of plaque-forming cells (PFC) developing against SRBC. The immune response was usually tested 5 days after immunization with SRBC, but the suppression persisted also at 8 days. Both direct and indirect PFC were suppressed to the same extent.

The immunosuppressive effect of the GVH reaction on the PFC response to SRBC was probably not due to a redistribution of the PFC from the spleen to other sites, because humoral antibody titres to SRBC as measured by agglutination and haemolysis, were equally suppressed (Table 2).

The immune response to SRBC is thymus dependent. In order to study whether the GVH reaction would also affect the immune response to non-thymus dependent antigens, analogous experiments were performed with *E. coli* endotoxin, which has been found to be independent upon the presence of thymus-derived lymphocytes (Möller, 1970). As can be seen from Table 1 the cellular immune response to *E. coli* endotoxin was also markedly suppressed.

TABLE 1

EFFECT OF GVH REACTIONS IN ADULT F_1 *RECIPIENTS ON CELLULAR ANTIBODY SYNTHESIS TO SRBC AND *E. coli* ENDOTOXIN

Experiment no.	Donor genotype	Recipient† genotype	Antigen (Time of injection)	PFC/10^6 spleen cells (mean† ± SE) at indicated days: Days	Direct PFC	Indirect PFC	Per cent inhibition by ‡GVH of: Direct PFC	Indirect PFC
1	A	A × C57L	SRBC (0)	5	2·62 ± 0·05		− 17·8	
	A × C57L	A × C57L	SRBC (0)	5	2·55 ± 0·04			
	A	A × C57L	SRBC (7)	12	0·46 ± 0·25		98·2	
	A × C57L	A × C57L	SRBC (7)	12	2·21 ± 0·07			
2	A	A × A.CA	SRBC (0)	5	1·98 ± 0·12		− 49·5	
	A × A.CA	A × A.CA	SRBC (0)	5	1·80 ± 0·04			
	A	A × A.CA	SRBC (7)	12	0·62 ± 0·28		97·2	
	A × A.CA	A × A.CA	SRBC (7)	12	2·18 ± 0·12			
3	A	A × CBA	SRBC (7)	12	2·69 ± 0·08	1·69 ± 0·09	44·9	93·3
	A × CBA	A × CBA	SRBC (7)	12	2·95 ± 0·11	2·86 ± 0·04		
	A	A × CBA	SRBC (7)	16	1·71 ± 0·09	2·88 ± 0·14	− 8·9	35·4
	A × CBA	A × CBA	SRBC (7)	16	1·67 ± 0·12	3·07 ± 0·11		
4	A	A × CBA	SRBC (7)	11	2·19 ± 0·09	2·13 ± 0·15	87·3	78·1
	A × CBA	A × CBA	SRBC (7)	11	3·09 ± 0·03	2·79 ± 0·16		
	A	A × CBA	SRBC (7)	14	1·18 ± 0·07	2·57 ± 0·32	− 7·8	59·3
	A × CBA	A × CBA	SRBC (7)	14	1·15 ± 0·08	2·97 ± 0·07		
5	A	A × 5M	SRBC (7)	12	0·98 ± 0·45		99·1	
	A × 5M	A × 5M	SRBC (7)	12	3·01 ± 0·03			
	A	A × 5M	*E.* tox (7)	12	0·58 ± 0·24		98·1	
	A × 5M	A × 5M	*E.* tox. (7)	12	2·50 ± 0·11			
6	A	A × CBA	SRBC (7)	12	0·51 ± 0·16		99·7	
	A × CBA	A × CBA	SRBC (7)	12	2·99 ± 0·10			
	A	A × CBA	*E.* tox. (7)	12	0·51 ± 0·19		97·6	
	A × CBA	A × CBA	*E.* tox. (7)	12	2·13 ± 0·29			
7	A	A × 5M	SRBC (7)	12	0·73 ± 0·21		99·0	
	A × 5M	A × 5M	SRBC (7)	12	2·71 ± 0·04			
	A	A × 5M	*E.* tox. (7)	12	1·70 ± 0·29		86·4	
	A × 5M	A × 5M	*E.* tox. (7)	12	2·57 ± 0·06			

* Five recipients per group. Each recipient received 50×10^6 donor spleen cells.

† $\log_{10}$ mean of the PFC.

‡ Percentage inhibition = PFC/10^6 cells in control (F_1 into F_1) minus PFC/10^6 in experimental group (P into F_1) in per cent of the control.

EFFECT OF THE GVH REACTION ON THE IMMUNE RESPONSE OF ADOPTIVELY TRANSFERRED IMMUNOCOMPETENT CELLS

Previous studies (Möller and Sjöberg, 1970) on antigenic competition between HRBC and SRBC showed that animals which were pretreated with one antigen (eg. HRBC) and thereafter irradiated and repopulated with immunocompetent cells failed to support antibody synthesis by the adoptively transferred cells, stimulated with another antigen (SRBC). Thus, antigenic competition appeared to make the animals unable to support antibody synthesis by adoptively transferred spleen cells to a non-cross-reacting antigen. Analogous studies were performed in the present test system. Thus, a GVH reaction was induced by transfer of parental spleen cells into untreated adult F_1 recipients. 7 days later the recipients were inoculated with parental spleen cells and at the same time immunized against SRBC. The F_1 recipients were either untreated or irradiated with 350 r prior to the adoptive transfer of parental spleen cells. The latter cells were derived either from normal animals or from animals immunized previously against SRBC. Irrespective of whether the recipients were irradiated or not and independent of the

TABLE 2
EFFECT OF GVH REACTIONS IN ADULT F_1 HYBRIDS* ON SERUM ANTIBODY SYNTHESIS AGAINST SRBC

Donor genotype	Recipient genotype	Time of injection of SRBC	Per cent inhibition by the GVH reaction of the PFC/10° cells		$\log_2$ antibody titres (mean ± SE) in	
			Direct PFC	Indirect PFC	Agglutination	Haemolysis
A	A×5M	7	94·2	98·1	2·5±0·9	7·0±1·3
A×5M	A×5M	7			6·8±0·2	11·8±0·2
A	A×A.CA	7	56·6	97·4	3·3±0·9	7·3±0·3
A×A.CA	A×A.CA	7			6·8±0·5	10·3±0·6

* Five recipients per group.

immune status of the adoptively transferred parental spleen cells, it was found that F_1 hybrids subjected to a GVH reaction failed to support adequately the cellular immune response to SRBC, carried out by adoptively transferred parental spleen cells (Table 3).

Analogous experiments to those described in Table 3 were performed with syngeneic F_1 instead of parental spleen cells. Thus, a GVH reaction was induced for 7 days as described before and thereafter F_1 spleen cells from animals hyperimmunized to SRBC were transferred to these recipients. The recipients were either left untreated or were irradiated with 350–600 r prior to transfer. Disregarding the treatment of the recipient, a clear suppression of the PFC response to SRBC was found (Table 4). In this test system, the untreated F_1 recipient had been subjected to a GVH reaction induced by the inoculation of parental cells, and it is likely that the GVH reaction would continue and affect the adoptively transferred spleen cells of F_1 genotype. However, in animals subjected to 600 r it is to be expected that the GVH reaction is prevented to a large extent. In spite of this the degree of immunosuppression was very pronounced. Thus, the immune response of adoptively transferred spleen cells of both F_1 and parental genotype was suppressed in recipients subjected to a GVH reaction.

TABLE 3

IMPAIRED CELLULAR ANTIBODY SYNTHESIS TO SRBC AFTER TRANSFER OF PARENTAL SPLEEN CELLS INTO 350 r IRRADIATED RECIPIENTS SUBJECTED TO A GVH REACTION SINCE 7 DAYS

Experiment no.	GVH conditions*		Immune status of transferred† parental cells	PFC/10^6 cells (mean‡ ± SE)§		Per cent inhibition	
	Donor	Recipient		Direct PFC	Indirect PFC	Direct PFC	Indirect PFC
1	A	A × 5M	Normal	0·46 ± 0·39	0·70 ± 0·47	69·5	95·2
	A × 5M	A × 5M		0·97 ± 0·16	2·05 ± 0·22		
2	A	A × 5M	Normal	0·45 ± 0·18	0·77 ± 0·19	86·7	99·1
	A × 5M	A × 5M		1·31 ± 0·19	2·79 ± 0·21		
3	A	A × 5M	Normal	0·44 ± 0·28	0·18 ± 0·18	69·3	99·1
	A × 5M	A × 5M		0·94 ± 0·19	2·23 ± 0·42		
4	A	A × 5M	5 days after first immunization	0·71 ± 0·36	0·40 ± 0·40	90·8	99·8
	A × 5M	A × 5M	with SRBC	1·75 ± 0·28	3·16 ± 0·18		
5	A	A × C57BL	5 days after first immunization	< 0·0	< 0·0	100	100
	A × C57BL	A × C57BL	with SRBC	2·5 ± 0·19	2·77 ± 0·10		

* The GVH reactions were initiated by the transfer of 50×10^6 spleen cells at day 0.
† At day 7 the recipients were irradiated with 350 r and thereafter given 10^7 parental spleen cells mixed with SRBC.
‡ The PFC response was determined 7 days after transfer.
§ $\log_{10}$ mean ± $\log_{10}$ SE.

TABLE 4

IMPAIRED CELLULAR ANTIBODY SYNTHESIS TO SRBC AFTER TRANSFER OF F_1 HYBRID SPLEEN CELLS INTO IRRADIATED OR UNIRRADIATED RECIPIENTS SUBJECTED TO A GVH REACTION SINCE 7 DAYS

Experiment no.	GVH conditions		X-ray dose to recipients of transfer	Immune status of transferred hybrid cells	PFC/10^6 cells (mean ± SE)*		Per cent inhibition	
	Donor	Recipient			Direct PFC	Indirect PFC	Direct PFC	Indirect PFC
1	A	A × 5M	—	Hyperimmune to SRBC	—	2·06 ± 0·25	—	49·1
	A × 5M	A × 5M	—	Hyperimmune to SRBC	—	2·35 ± 0·18		
2	A	A × 5M	—	Hyperimmune to SRBC	—	< 0·0		100
	A × 5M	A × 5M	—	Hyperimmune to SRBC	—	1·84 ± 0·17		
3	A	A × 5M	—	Hyperimmune to SRBC	—	< 0·0		100
	A × 5M	A × 5M	—	Hyperimmune to SRBC	—	2·68 ± 0·07		
4	A	A × 5M	350 r	6 days after first	0·1 ± 0·10	< 0·0	99·2	100
	A × 5M	A × 5M	350 r	challenge with SRBC	2·26 ± 0·24	3·04 ± 0·58		
5	A	A × C57L	600 r	Hyperimmune to SRBC	1·61 ± 0·58	3·25 ± 0·20	79·8	34·8
	A × C57L	A × C57L	600 r	Hyperimmune to SRBC	2·31 ± 0·33	3·43 ± 0·15		

* $\log_{10}$ mean ± $\log_{10}$ SE.

GVH REACTIONS INDUCED BY PARENTAL CELLS SENSITIZED TO SRBC

The previous findings suggest that a GVH reaction of 7 days duration changes the environment in the recipients to such an extent that the adoptively transferred cells cannot adequately respond to SRBC. Therefore, it would be expected that induction of GVH reaction by parental spleen cells already presensitized to SRBC would also result in a decreased immune response to SRBC injected 7 days later.

TABLE 5
CELLULAR ANTIBODY SYNTHESIS TO SRBC IN F_1 HYBRIDS SUBJECTED TO A GVH REACTION FOR 7 DAYS BY THE INOCULATION OF PARENTAL SPLEEN CELLS FROM DONORS SENSITIZED TO SRBC

Experiment no.	GVH conditions*		PFC/10^6 cells (mean ± SE)†		Per cent inhibition	
	Donor	Receipient	Direct PFC	Indirect PFC	Direct PFC	Indirect PFC
1	5M	A × 5M	1·27 ± 0·10	1·52 ± 0·05	95·5	95·6
	5M	5M	2·62 ± 0·32	2·88 ± 0·16		
2	5M	A × 5M	1·01 ± 0·33	1·05 ± 0·54	95·9	99·5
	5M	5M	2·39 ± 0·09	3·39 ± 0·09		
3	C57BL	A × C57BL	1·23 ± 0·22	1·95 ± 0·22	53·3	91·7
	C57BL	C57BL	1·56 ± 0·17	3·03 ± 0·27		
4	5M	A × 5M	2·08 ± 0·11	2·65 ± 0·14	63·8	84·5
	5M	5M	2·52 ± 0·07	3·46 ± 0·15		
5	C57L	A × C57L	1·28 ± 0·11	1·68 ± 0·22	88·4	96·3
	A × C57L	A × C57L	2·16 ± 0·13	3·11 ± 0·09		
6	C57L	A × C57L	1·78 ± 0·43	2·14 ± 0·13	−2230	26·8
	A × C57L	A × C57L	0·42 ± 0·21	2·28 ± 0·08		
7	CBA	A × CBA	2·54 ± 0·09	3·57 ± 0·04	−342	−77·7
	CBA	CBA	1·89 ± 0·09	3·32 ± 0·05		
8	A	A × C57L	2·35 ± 0·06	3·31 ± 0·04	−6·7	−62·9
	A	A	2·32 ± 0·03	3·10 ± 0·09		
9	A	A × C57BL	3·72 ± 0·02		−1745	
	A × C57BL	A × C57BL	2·45 ± 0·12			
10	A	A × CBA	3·26 ± 0·27	4·31 ± 0·19	−298·2	−168·9
	A × CBA	A × CBA	2·66 ± 0·14	3·88 ± 0·05		
11	CBA	A × CBA	1·98 ± 0·07	3·22 ± 0·08	−90·2	−18·7
	A × CBA	A × CBA	1·70 ± 0·05	3·14 ± 0·11		
12	CBA	A × CBA	2·49 ± 0·13	3·96 ± 0·16	−346·0	−623·1
	A × CBA	A × CBA	1·84 ± 0·07	3·10 ± 0·22		
13	A	A × 5M	1·81 ± 0·21	2·94 ± 0·07	−29·2	−33823
	A × 5M	A × 5M	1·69 ± 0·15	0·41 ± 0·37		
14	CBA	A × CBA	3·12 ± 0·25	3·61 ± 0·10	−46·8	−30·3
	A × CBA	A × CBA	2·96 ± 0·11	3·49 ± 0·15		

* 50×10^6 parental or F_1 hybrid spleen cells from donors hyperimmunized to SRBC were inoculated i.v. into untreated adult F_1 hybrids. 7 days later the recipients were given 4×10^8 SRBC i.v. Each group consisted of five animals.
† The PFC response to SRBC was determined 5 days after immunization with SRBC. All values are expressed as $\log_{10}$.

This prediction could be verified in certain strain combinations, but not in others. Thus, a GVH reaction induced by C57L, C57BL or B10.5M spleen cells from animals immunized to SRBC, invariably resulted in a decreased number of PFC to SRBC given 7 days after induction of the GVH reaction (Table 5). This was true whether the comparison was made between (1) the same parental cell suspension injected into either F_1 hybrids or into parental mice and the recipient subsequently challenged with SRBC or (2) F_1 recipients receiving either parental or F_1 hybrid cells.

However, in analogous experiments performed with strain A or CBA spleen cells there was no suppressive effect on the PFC response to SRBC, whether the comparison was made according to alternatives (1) or (2) listed above (Table 5). Actually, there was often a marked degree of stimulation of the immune response to SRBC in animals subjected to GVH reactions.

ANTIGEN-SENSITIVE CELLS IN ANIMALS SUBJECTED TO GVH REACTIONS

The previous results demonstrate that GVH reaction initiated by non-sensitized parental spleen cells induces a pronounced immunosuppressive effect on the primary immune response to SRBC, provided it was induced 7 days earlier. Furthermore, these animals constitute a poor environment for the immunological capacity of adoptively transferred spleen cells of either parental or F_1 genotype, even if the donor cells are presensitized to SRBC. However, a GVH reaction induced with parental cells already sensitized to SRBC results in suppression of the secondary anti-SRBC response only when the donor cells are of $H\text{-}2^b$ genotype, whereas there is no effect or a stimulating effect when the donors are of $H\text{-}2^a$ and $H\text{-}2^k$ genotypes.

Previous studies on antigenic competition between SRBC and HRBC showed that the number of antigen-sensitive cells sensitive to the suppressed antigen was normal (Möller and Sjöberg, 1970). Analogous studies were performed in this case. Two systems were employed. (1) The F_1 recipients were presensitized to SRBC and 7 days after the induction of a GVH reaction the spleens of the recipients were taken out and transferred into irradiated F_1 hybrids, which were thereafter stimulated with SRBC. This is a test for the number of antigen-sensitive cells in the recipients. (2) The parental donor cells were derived from animals immunized to SRBC and 7 days after their inoculation into untreated F_1 recipients, the spleens of the hosts were transferred to irradiated mice together with SRBC to test the number of antigen-sensitive cells of parental origin.

As can be seen in Table 6 there was a decreased number of antigen-sensitive cells of F_1 hybrid origin as a consequence of the GVH reaction (Experiments 1–5). However, the decrease was comparatively small and not as pronounced as the previously demonstrated inhibition of the primary immune response of adoptively transferred cells. This inhibiting effect is to be expected, because the inoculated parental cells will most probably kill some of the spleen cells in the F_1 hybrid recipients in the course of the GVH reaction.

Analogous studies were performed with spleen cells from F_1 hybrid recipients which 7 days earlier had been given parental spleen cells from animals immunized to SRBC. (Table 6, Experiments 6–15). As can be seen from Table 6 there was little or no suppression of the immune response to SRBC in the secondary irradiated host when the parental strains were of strain A or CBA genotype (Experiments 6–10). Usually there was evidence of an increased immune response. However, when the donor cells were of $H\text{-}2^b$ genotype there was clearly a decreased number of antigen-sensitive cells (Experiments 11–15), although the degree of the suppression of the response was not as marked as when the primary immune response to SRBC of these animals was studied.

Thus, when the number of antigen sensitive cells of F_1 hybrid origin was studied they were suppressed in number as a consequence of the GVH reaction, although the degree of suppression was smaller than that caused by GVH reaction on the primary response. When antigen-sensitive cells of the donor genotype was investigated there was no evidence for a decreased number of antigen-sensitive cells to SRBC when strain A or CBA was used,

TABLE 6
EFFECT OF GVH REACTIONS ON ANTIGEN-SENSITIVE CELLS TO SRBC*

Experiment no.	GVH conditions*				Transfer to 350 r irradiated	PFC/10^6 spleen cells (mean† ± SE) in recipients			
	Donor		Recipient						
	Strain	Immune status	Strain	Immune status		Direct PFC	Per cent inhibition‡	Indirect PFC	Per cent inhibition
1	A	Normal	A × C57BL	Imm. SRBC	A × C57BL	3·09 ± 0·09	54·3	4·41 ± 0·05	30·2
	A × C57BL	Normal	A × C57BL	Imm. SRBC	A × C57BL	3·43 ± 0·18		4·57 ± 0·10	
2	A	Normal	A × 5M	Imm. SRBC	A × 5M	3·20 ± 0·12	84·0	3·94 ± 0·12	76·3
	A × 5M	Normal	A × 5M	Imm. SRBC	A × 5M	3·99 ± 0·07		4·56 ± 0·09	
3	A	Normal	A × C57L	Imm. SRBC	A × C557L			3·99 ± 0·22	25·2
	A × C57L	Normal	A × C57L	Imm. SRBC	A × C57L			4·11 ± 0·17	
4	CBA	Normal	A × CBA	Imm. SRBC	A × CBA			2·83 ± 0·22	94·7
	A × CBA	Normal	A × CBA	Imm. SRBC	A × CBA			4·11 ± 0·17	
5	A	Normal	A × CBA	Imm. SRBC	A × CBA	3·13 ± 0·06	33·9	4·54 ± 0·57	14·5
	A × CBA	Normal	A × CBA	Imm. SRBC	A × CBA	3·31 ± 0·10		4·61 ± 0·15	
6	A	Imm. SRBC	A × C57BL	Normal	A × C57BL	1·71 ± 0·26	−143·0	2·42 ± 0·13	−181·7
	A × C57BL	Imm. SRBC	A × C57BL	Normal	A × C57BL	1·32 ± 0·26		1·97 ± 0·09	
7	A	Imm. SRBC	A × C57BL	Normal	A × C57BL	1·43 ± 0·19	50·4	2·71 ± 0·08	−1·151
	A × C57BL	Imm. SRBC	A × C57BL	Normal	A × C57BL	1·74 ± 0·14		1·61 ± 0·07	
8	CBA	Imm. SRBC	A × CBA	Normal	CBA	1·32 ± 0·09	−133·3	2·80 ± 0·14	−78·1
	A × CBA	Imm. SRBC	A × CBA	Normal	A × CBA	0·96 ± 0·18		2·55 ± 0·08	
9	CBA	Imm. SRBC	A × CBA	Normal	CBA	1·41 ± 0·41	81·6	1·74 ± 0·72	92·6
	A × CBA	Imm. SRBC	A × CBA	Normal	A × CBA	2·15 ± 0·21		2·87 ± 0·17	
10	CBA	Imm. SRBC	A × CBA	Normal	CBA			3·38 ± 0·06	−87·5
	A × C57L	Imm. SRBC	A × C57L	Normal	A × C57L			1·34 ± 0·10	
	CBA	Imm. SRBC	CBA	Normal	CBA			3·11 ± 0·06	21·4
11	C57L	Imm. SRBC	A × C57L	Normal	A × C57L			1·43 ± 0·09	
	A × C57L	Imm. SRBC	A × C57L	Normal	A × C57L			1·54 ± 0·10	
12	5M	Imm. SRBC	A × 5M	Normal	5M			1·43 ± 0·13	73·8
	5M	Imm. SRBC	5M	Normal	5M			2·01 ± 0·30	
13	C57L	Imm. SRBC	A × C57L	Normal	C57L			1·66 ± 0·26	9·5
	C57L	Imm. SRBC	C57L	Normal	C57L			1·62 ± 0·08	
14	5M	Imm. SRBC	A × 5M	Normal	5M			2·42 ± 0·16	53·8
	5M	Imm. SRBC	5M	Normal	5M			2·75 ± 0·10	
15	5M	Imm. SRBC	A × 5M	Normal	5M			1·19 ± 0·13	86·2
	5M	Imm. SRBC	5M	Normal	5M			2·07 ± 0·09	

* The GVH reaction was induced by the i.v. inoculation of 50×10^6 spleen cells. 7 days later the spleens of these animals were removed and the cells mixed with SRBC and inoculated into irradiated (350 r) recipients. The number of PFC against SRBC in these recipients was determined 7 days later.
† $\log_{10}$ mean ± SE.
‡ Per cent inhibition defined as in previous Tables.

whereas a decreased number was found when spleen cells of $H\text{-}2^b$ genotype were employed, in analogy with the previous results. However, the decreased number of antigen-sensitive cells in the $H\text{-}2^b$ system was not as marked as the degree of immunosuppressive effect of the primary or secondary response. Thus, the GVH reaction appears to suppress the productive phase of the immune response to a larger extent than it affects the number of antigen-sensitive cells to SRBC.

DISCUSSION

The phenomenon of antigenic competition has been used as an argument for the existence of multipotent antigen-sensitive cells. However, recent studies by Radovich and Talmage (1967), Brody and Siskind (1969) and Möller and Sjöberg (1970) made this assumption unlikely. It appeared more likely that antigenic competition was caused by non-specific factors, such as competition for some limiting factors, some aspects of antigen processing or localization or by the production of substances inhibiting the immune response. The last possibility received some support from the finding that pretreatment of mice with HRBC made the animals incompetent to support antibody production to an unrelated antigen by adoptively transferred spleen cells (Möller and Sjöberg, 1970). Presumably one antigen changed the environment in the host in such a way that adoptively transferred immunocompetent cells, even when they were presensitized to the second antigen became inactivated. Based on these studies it was suggested that antigen-stimulated thymus-dependent lymphocytes become changed in such a way that they indirectly affected other lymphocytes. This effect could either be due to the release of a humoral substance acting locally and tending to change the thymus-derived lymphocytes in such a way that they could no longer effectively collaborate with bone-marrow cells in the induction of the immune response to an unrelated antigen, or else the hypothetical factors affected directly the bone marrow lymphocytes, presumably by suppressing their cell division.

The graft versus host reaction has been used as a model for antigenic competition (Lawrence and Simonsen, 1967). By itself the GVH creates certain problems, because the number of antigen reactive cells sensitive to histocompatibility antigens determined by the 'strong' locus of the species is unusually large. This led Lawrence and Simonsen (1967) to postulate the existence of pluripotent cells, and the suppressive effect of a GVH reaction on the immune response to SRBC was interpreted in these terms. Our experimental findings are in agreement with those of Lawrence and Simonsen (1967). However, they inoculated parental cells into irradiated recipients and found that the transferred parental cells lost their ability to produce antibodies to SRBC after 7–10 days residence in the hybrids. In the present experiments parental cells were transferred into *untreated* F_1 hybrids. In such a situation both the transferred cells and the normal hybrid cells would be expected to respond to SRBC. As reported above, the response to SRBC was markedly suppressed. Although the failure of the recipient lymphocytes to respond to SRBC could be due to a toxic effect caused by the GVH reaction, the failure of adoptively transferred parental cells to produce antibodies to SRBC made this possibility unlikely. Thus, in analogy with the previous findings on antigenic competition between SRBC and HRBC (Möller and Sjöberg, 1970), it appeared likely that a GVH reaction changed the environment in the recipient in such a way that it could not adequately support antibody synthesis by transferred normal cells. Analogous experimental findings were made previously by

Celada and Welshons (1962) but were interpreted in a different way. Thus, they used the findings as evidence for the existence of recessively determined antigens present in the parental cells, but absent from the hybrid cells. This interpretation cannot be correct because transfer of F_1 hybrid cells into irradiated F_1 recipients, subjected to a GVH reaction resulted in suppression of the immune response to SRBC to the same extent as when parental cells were transferred.

When the GVH reaction was initiated by cells already sensitized to SRBC the degree of immunosuppression seemed to depend on the genotype of the cell donor. It would be expected from the previous results on adoptive transfer of cells that immune (to SRBC) parental cells inducing a GVH reaction for 7 days would cause suppression of the immune response to SRBC. However, this was not found to be true when the parental cells were of A or CBA (*H*-2^a or *H*-2^k) genotypes. On the other hand, when the transferred cells belonged to strains C57L, C57BL or B10.5M, a marked degree of suppression was found. All these strains belong to the *H*-2^b genotype. This is analogous to the findings by Boyse (1959), Cudkowicz and Stimpfling (1964) and others showing that lymphoid cells and bone marrow cells of *H*-2^b genotype do not survive well in F_1 hybrids. It was initially demonstrated by Boyse (1959) that heterologously sensitized C57BL spleen cells were rapidly lost in A × C57BL recipients, whereas strain A cells persisted. Therefore, the present results support the previous finding that *H*-2^b cells do not survive in F_1 hybrids, the mechanism for this being unknown as yet.

In view of these findings it would be expected that the number of antigen sensitive cells present in an animal subjected to a GVH reaction would depend on the genotype of the donor cells. This was also found to be true. Thus, there was a marked decline of the number of antigen sensitive cells of parental type belonging to *H*-2^b genotype, whereas there was no evidence for a decreased number of such cells when other genotypes were studied. However, when antigen-sensitive cells of F_1 genotype were studied in an analogous system, it was shown that they were decreased, although to a small extent as compared to the marked degree of immunosuppression found when the animals were immunized directly to SRBC.

The present findings are in general agreement with those reported previously with SRBC and HRBC (Möller and Sjöberg, 1970) with the additional complexities created by the GVH reaction. Thus, it would appear that the reaction of the parental cells towards the histocompatibility antigens in the F_1 hybrid recipients causes some changes of the environment, making both the host cells and the transferred cells incompetent to respond to a non-cross-reacting antigen (SRBC), provided the GVH reaction has been initiated at least 7 days previously. The mechanism(s) responsible for suppression were resistant to irradiation and affected also adoptively transferred cells.

Studies of DNA synthesis (Möller, 1970) in spleen cells of intact animals during antigenic competition supports the concept that red cell antigens result in a marked suppression of DNA synthesis in the spleen. In view of previous findings (Möller and Sjöberg, 1970) it is suggested that antigenic competition is caused by suppressed proliferation of immunocompetent cells and not to an effect on initial number of antigen-sensitive cells. It seems plausible that the introduction of one antigen stimulates thymus-dependent lymphocytes. These lymphocytes could affect other thymus-derived lymphocytes by releasing various humoral substances so that they become incompetent to co-operate with bone marrow cells. Alternatively, such factors could directly suppress proliferation of bone-marrow cells. There is evidence that lymphocytes which have been in contact with one antigen lose

their ability to respond to this antigen and also to other antigens (Möller, 1970). Thus, pretreatment of lymphocytes for 3 days *in vitro* with low doses of antigen (PPD) suppresses their ability to respond to an optimal dose of the same antigen and also to optimal doses of other antigens.

The present *in vivo* findings have been substantiated in an analogous antigenic system by *in vitro* studies on the primary immune response to SRBC (Sjöberg, unpublished data).

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