THE INFLUENCE OF ALLOGENEIC OR SYNGENEIC CELLS SURFACE BACKGROUNDS ON THE ANTIBODY RESPONSE OF MICE TO RABBIT Fab' FRAGMENTS

by

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In the Department of Microbiology

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

February, 1977

Robert Bruce Acres, 1977
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ABSTRACT

Recent work has shown that in vitro, the cytotoxic immune response to cell surface antigens is enhanced if the antigen to which the immune response is directed, is on cells bearing major histocompatibility antigens identical to those of the responding cells. This 'H-2 restriction' has been demonstrated in the mouse using virally infected cells, haptenated cells, cells bearing the male Y antigen, and cells differing at the minor histocompatibility loci.

Other investigations have shown that antigenic determinants coupled to tolerated antigens or isologous serum proteins, elicit a humoral response which is weaker than that to the same determinant coupled to a heterologous carrier. This and other evidence suggest an inverse relationship between humoral and cell mediated immunity.

The purpose of this investigation was to explore the humoral response to antigens on cells which are syngeneic or allogeneic to the recipient, in order to determine the influence of a tolerated as opposed to allogeneic background.

The approach used in this study was as follows: Mice were immunized with antigen (rabbit Fab' fragments) attached to syngeneic, allogeneic, or F₁ (semi syngeneic), irradiated spleen cells. Specific anti-rabbit Fab' plaque forming cell numbers were determined five days after the third, weekly injection of Fab' coated spleen cells. Some of the spleen cells taken from the responding animals, on the day of sacrifice, were incubated in vitro with soluble antigen (rabbit Fab' fragments not specific for mouse cells) for four days. The results showed that the humoral response to antigens attached to cells bearing 'self' histocompatibility antigens (i.e. syngeneic or F₁ semi syngeneic cells) was
significantly weaker than the humoral response to the same antigen on allogeneic cells. The effect of \textit{in vitro} incubation of responder spleen cells for four days with soluble antigen was to reverse this difference. Those spleen cells exhibiting lowered plaque forming cell numbers initially (i.e. those cells from mice immunized with antigen on syngeneic or \textit{F}_1 cell surfaces) showed, after incubation, a response equal to or greater than those cells which initially (before \textit{in vitro} incubation) demonstrated a larger response (i.e. cells from those mice immunized with antigen on allogeneic cell surfaces).
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I would like to thank Dr. Julia Levy for her encouragement, suggestions, and unending patience during the research for, and preparation of this thesis.
ABBREVIATIONS

Abbreviations used in this thesis are:

- MHC: major histocompatibility complex
- H-2: that region of the mouse genome encoding the MHC antigens and the immune response genes
- BSA: bovine serum albumin
- Fab': approximately 1/3 of the immunoglobulin molecule which does not fix complement and contains 1 antigen binding site
- CFA: complete Freund's adjuvant
- PBS: phosphate buffered saline
- DEAE: diethyl amino ethyl
- SRBC: sheep red blood cells
- PFC: plaque forming cells
- DPM: disintegrations per minute
- HBSS: Hank's balanced salt solution
- EDCI: 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide
- LPS: lipopolysaccharide
- $^3$H: Tritium
- CPM: counts per minute
INTRODUCTION

The role of the genes of the major histocompatibility complex (MHC) of mice in the generation of various immunological responses has been studied intensively in the past few years. It has been shown that cooperation between B and T cells occurs only when they share at least one set of H-2 antigens (1-4). More recently, it has been shown that in order for virus immune T cells to generate an in vitro cytotoxic reaction to virally infected cells, it is necessary for the effector and target cells to share either the K or D regions of the MHC (5-7). This latter aspect of H-2 restriction also applies to chemically modified (haptenated) target cells (8-10), those exhibiting minor histocompatibility antigens (11), or the male Y antigen (12). All of these observations imply the importance of recognition of H-2 gene products in the interaction of lymphocytes and target cells in the generation of cytotoxic T cells.

Recent observations of Trinchieri et al (13) however, indicate there may be an inverse relationship between the generation of cytotoxic T cells and a humoral response to virus antigens. Using SV-40 transformed human-mouse fusion cell products, these investigators found that while H-2 restriction of cell-mediated immunity prevailed, the anti-viral antibody titre was highest in those mice not showing the presence of cells cytotoxic for SV-40 transformed human cells. This inverse relationship has also been demonstrated with such antigens as tuberculin (Janichi et al 1970), lysozyme (Thompson et al 1972) and chemically modified antigens (Parish 1972).

That the humoral response to antigens may be inhibited or suppressed by the association of the antigen with tolerated antigens has been demonstrated with a number of systems. Scibieni et al (14) reported that
mice, neonatally tolerized to lysozyme and subsequently challenged with lysozyme covalently linked to bovine serum albumin (BSA), showed a markedly suppressed response to BSA when compared to the response of lysozyme tolerant animals immunized with a mixture of BSA and lysozyme (not linked). Similarly, Lee and Sehon (15) found that mice challenged with haptenated, self serum proteins (epitope density of 9, 11 or 12 haptens per carrier molecule) showed a suppressed anti-hapten response when compared to the response in animals immunized with the hapten conjugated to a foreign immunogenic protein.

These apparently unrelated observations raise some interesting questions regarding the relationship of humoral vs. cell-mediated immune responses, and the role of self or non-self markers on cells with which new or non-self antigens are associated. The work reported in this thesis was designed to investigate this relationship. Essentially, I studied the humoral response in mice to an antigen (rabbit Fab' fragments) which was presented during immunization in association with irradiated lymphoid cells of syngeneic, allogeneic or F1 origin. Since H-2 restriction, as measured so far, has only involved studies on the generation of cytotoxic T cells, it was felt that an analogous study on the generation of helper T cells and antibody forming cells would help to complete the understanding of the role of MHC in immune responsiveness. An understanding of the influence of self-vs.-non-self backgrounds on immune responsiveness to membrane associated antigens of either viral and/or neoplastic origins may have broad significance in a number of disease conditions.
MATERIALS AND METHODS

Preparation of the Test Antigens

Rabbit Fab' fragments (R.Fab') with specificity for either sheep erythrocytes (SRBC) or cell surface antigens of lymphocytes from various mouse strains (C57 Bl/6J, DBA/2J and CBA/J) were prepared as follows:

A 50% suspension of SRBC in phosphate buffered saline (PBS) was emulsified with 25% (v/v) of Complete Freund's Adjuvant (CFA). A total volume of 1.0 ml was injected in 4 sites intramuscularly, and one intraperitoneally into rabbits. Animals were boosted at monthly intervals with 1.0 ml of 50% SRBC in PBS administered at first intravenously then intramuscularly, and bled one week after injection. Initial immunization with splenic mouse lymphocytes was with $10^8$ cells in PBS and 25% CFA administered as above. Subsequent immunizations were carried out with $10^8$ lymphocytes in PBS given intramuscularly at monthly intervals with blood being taken 1 week following injection. The immunoglobulin fraction of the immune serum was prepared according to standard methods with ammonium sulfate precipitation (40% saturation) followed by dialysis and ion exchange chromatography on DEAE cellulose (16). Fab' fragments were prepared by papain digestion according to the method of Porter (17). The Fab' fragments with specificity for mouse cell surface antigens was used to bind to irradiated mouse lymphocytes which were subsequently used for immunization of recipients. The Fab' fragments specific for SRBC were used to coat these cells for use in the plaque assay.
**Immunization**

Mouse splenic lymphocytes to be used for immunization were prepared by passage of clumps of cells through a stainless steel 50 gauge mesh in PBS containing 5% foetal calf serum (FCS). The cells were treated with .83% NH₄Cl in 5% FCS for 4 min and centrifuged at 200 x g to remove erythrocytes, and washed twice in PBS containing 5% FCS. These cells were irradiated with 2000 rads on a Gammacell 220, Atomic Energy of Canada Ltd., and were incubated with appropriate specific R.Fab' fragments at a concentration of 0.5 mg per 10⁷ spleen cells per ml in Hepes buffered 1640 medium (Grand Island Biological Co.) containing 5% FCS. The coated cells were washed three times in saline and suspended at a concentration of 10⁸ cells per ml. Cells were counted by the trypan blue exclusion method.

Recipient mice were injected intraperitoneally with 10⁷ of the R.Fab' coated lymphocytes at weekly intervals, sacrificed 5 days after the last injection, and their spleens used for assay of plaque forming cells (PFC) using R.Fab' coated SRBC as the antigen.

The amount of R.Fab' attached to the immunizing mouse lymphocytes was quantitated as follows. An aliquot of the R.Fab' fragments to be used was iodinated with ¹²⁵I by the chloramine T method (18) and the specific activity determined per mg protein, using CPM measured on a Beckman Biogamma counter and an extinction coefficient for the Fab' fragments as O.D.₂⁸₀ 1.5 mg protein ml⁻¹. Known proportions of tracer ¹²⁵I labelled R.Fab' were admixed with unlabelled R.Fab' during the incubation with the irradiated lymphocytes. Subsequently, the amount of radioactivity recovered on the labelled cells was assumed to be proportional to the relative amount of unlabelled R.Fab' fragments attached. In all
instances, regardless of the strain of mouse lymphocytes used, it was found that 10 μg of Fab' + 10% was bound to $10^7$ lymphocytes. Thus it was assumed that each injection of R.Fab' coated cells into recipient mice constituted immunization with 10.0 μg of R.Fab' fragments.

Mouse kidney cells were originally used for immunization, but, due to poor cell survival and tedious isolation procedures, they were abandoned in favour of heartier and more easily obtainable spleen cells. Isolation of kidney cells was carried out as follows: Mouse kidneys were cut into small pieces and suspended in prewarmed (37°C) 0.25% trypsin in Hank's balanced salt solution (8 to 10 kidneys to 50 ml of HBBS). This suspension was stirred for 1 hour at 37°C, the large pieces allowed to settle, and the supernatant containing the cells pipetted off. The large pieces were then resuspended in fresh 0.25% trypsin in HBBS and the procedure repeated, until only white connective tissue was left in the suspension. The cells in the supernatant were centrifuged and washed in PBS, treated with 0.83% NH$_4$Cl for 3 minutes, to remove erythrocytes, washed again, and layered on top of 8 ml FCS and centrifuged at 500 rpm in order to remove pieces of kidney still remaining. Survival rate was roughly $10^5$ cells per kidney.

**Chemical Attachment of Antigen to Cells**

1. Coupling with 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide HCl (EDCI):

Rabbit Fab' fragments prepared from rabbits immunized with SRBC were attached to mouse kidney or spleen cells with EDCI. Rabbit red blood cells were used to determine which concentration of EDCI would
give maximum coupling with the least cell damage. Maximum attachment with the least lysis was obtained with 12 mg per ml EDCI and 1 mg per ml Fab'. However, it was discovered that after attachment of Fab' to spleen cells with EDCI, and washing with PBS, most cells absorbed trypan blue, indicating cell death, and that those cells which did survive were somewhat rough around the edges. Also, it was shown that EDCI is a hapten itself (Dale Gregerson, personal communication). For these reasons, the method of attachment was abandoned.

Other chemical fixatives which were tested include: Glutaraldehyde, which left cells alive and intact, but attached Fab' poorly; and several maleimides, all of which were insoluble in physiological saline.

**Plaque Assay**

Immunized animals were sacrificed 5 days after their last injection and their spleen cells used to assay the number of antibody forming cells (AFC) capable of responding to R.Fab' fragments. The microscope slide assay described by Cunningham (19) was used. SRBC were coated with anti-SRBC R.Fab' fragments by incubation of a 25% suspension of SRBC in PBS with the R.Fab' fragments at a concentration of 0.5 mg per ml at 37° for 1 h. The coated SRBC were washed three times and used in the assay. PFC assays for IgG-producing cells from each mouse were run in triplicate with the coated SRBC and SRBC alone. The number of specific anti-rabbit Fab' PFC was calculated by subtracting the background plaques found with SRBC alone, from the number with the R.Fab' coated SRBC. The reaction mixture for the PFC assay contained the following materials: 0.3 ml of Hepes buffered 1640 medium plus 5% FCS containing 5 x 10^6 mouse spleen lymphocytes, 0.1 ml of 20% SRBC (coated or uncoated), 0.05 ml of guinea
pig serum as a source of complement (the serum had been previously absorbed with R.Fab' coated SRBC and mouse spleen lymphocytes), and 0.05 ml of a 1/20 dilution of rabbit anti-mouse Ig serum. Each plaque chamber holds 0.1 ml of mixture, therefore $10^6$ mouse spleen cells. Plaques were allowed to develop at $37^\circ$ for 1½ h.

**In vitro Cell Culture**

Aliquots of spleen lymphocytes, taken from immunized mice 5 days after their last injection and assayed immediately for PFC, were also cultured in vitro and tested for the presence of PFC. Culturing procedures have been described previously (20). Briefly, $1 \times 10^7$ lymphocytes were cultured in 35 mm Petri dishes (Falcon No. 3301) in RPMI 1640 medium containing 10% FCS (Microbiological Associates No. 84557, Bethesda, Md.) and $5 \times 10^{-5}$ M 2-mercaptoethanol. Cells were cultured for 4 days in the presence of varying concentrations of R.Fab'. When they were harvested, they were tested for the presence of PFC for R.Fab' fragments as described above.

**Experimental Animals**

Female mice of the strains C57B1/6J, DBA/2J, CBA/J and C57B1/6J x DBA/2J F_1 (B_6D_2F_1) between 2 and 4 months of age were used in the following experiments. They were obtained from the Jackson Laboratories (Bar Harbor, Maine).

Outbred albino rabbits were used for the preparation of Fab' fragments of various specificities.
In vitro Mitogen and Antigen Stimulation

Spleen cells were prepared in phosphate buffered saline (PBS) + 5% fetal calf serum (FCS). The cells were centrifuged at 200 $\times$ g for 5 minutes, resuspended in PBS + 5% FCS, and the viability determined by trypan blue exclusion. Desired numbers of cells were centrifuged and resuspended in RPMI 1640 and dispensed into microtitre plates (Linbro Chemical Co., New Haven, Conn.). Each well contained $5 \times 10^5$ viable spleen cells in a final volume of 0.25 ml medium with FCS at a final concentration of 2%, and 5 $\mu$g concanavalin A, 6.25 $\mu$g lipopolysaccharide, or a range of concentrations of soluble rabbit Fab'. The cultures were incubated in a humidified atmosphere of 95% air - 5% CO$_2$ for 4 days. Eighteen hours before the cells were harvested, 1.0 $\mu$Ci of $^3$H-thymidine (specific activity 5.0 Ci/mmole, New England Nuclear, Montreal, Canada) was added to each well. The cells were harvested onto glass fibre filter paper, dried, and the radioactivity determined on a Nuclear Chicago Unilux II scintillation counter.
RESULTS

The time course of the response of C57 Bl/6 mice immunized with R.Fab' fragments attached to either syngeneic or allogeneic (DBA/2J) irradiated lymphocytes is shown in Fig. 1. It can be seen that the response at all times tested, of those mice immunized with R.Fab' fragments attached to allogeneic cells was higher in terms of PFC, and after the third injection, the differences were significant. Because the responses were maximal after the third injection subsequent experiments were carried out at this time. In order to determine the reproducibility of this observation and to observe the effect of a mixture of allogeneic and syngeneic cell markers on the immunizing cells, several experiments were performed in C57B1/6 mice immunized three times with R.Fab' labelled lymphocytes from syngeneic (C57B1/6), allogeneic (DBA/2J) and F₁ (B₆D₂F₁) animals. The results in Table 1 show that the responses of animals immunized with both syngeneic and F₁ labelled cells were significantly lower than those of mice immunized with allogeneic labelled cells. If this difference were attributable to the previously observed allogeneic effect (21), one might expect that the anti-SRBC response in the PFC controls would be higher and that the F₁ cells would generate a similar effect. This does not appear to be the case (Table 1). It would appear, rather, that the presence of 'self' antigens on the labelled cells has a negative effect on the generation of PFC to R.Fab' fragments.

In order to determine whether this observation was strain specific, another series of experiments was run with CBA mice immunized with either syngeneic (CBA) or allogeneic (DBA or C57B1/6) lymphocytes labelled with R.Fab' fragments. Analogous results were obtained in these instances (Table 2).
Figure 1

Time-course study of C57B1/6J mice immunized at weekly intervals with $10^7$, Fab' coated, irradiated spleen cells of C57B1/6J or DBA/2J origin. Recipient spleen cells were assayed for anti-R.Fab' PFC 5 days after each weekly injection. Results are expressed as anti-R.Fab' PFC per $10^7$ recipient spleen cells and represent the net total of anti-Fab' plaques after subtraction of spontaneous anti-SRBC plaques. Each point represents the average of 6 mice.

- R.Fab' coated C57B1/6J cells injected
- R.Fab' coated DBA/2J cells injected
Table 1: Immune response of C57B1/6 mice to Rabbit Fab' presented on DBA/2J, C57B1/6 or B_6.D_2.F_1 cells. All numbers represent PFC per spleen ± S.E.M. P = <0.02 according to Student's t test. (combined allogeneic vs. combined syngeneic and combined allogeneic vs. combined F_1). Each number represents the average of 3 mice.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>R.Fab' coated cells injected</th>
<th>Anti R.Fab' PFC/spleen</th>
<th>Anti-SRBC PFC/spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DBA/2J</td>
<td>24675 ± 8937</td>
<td>177 ± 57</td>
</tr>
<tr>
<td></td>
<td>C57B1/6</td>
<td>5298 ± 1744</td>
<td>150 ± 46</td>
</tr>
<tr>
<td></td>
<td>B_6.D_2.F_1</td>
<td>5458 ± 1914</td>
<td>216 ± 21</td>
</tr>
<tr>
<td>2</td>
<td>DBA/2J</td>
<td>11611 ± 2231</td>
<td>63 ± 62</td>
</tr>
<tr>
<td></td>
<td>C57B1/6</td>
<td>4201 ± 1442</td>
<td>33 ± 17</td>
</tr>
<tr>
<td></td>
<td>B_6.D_2.F_1</td>
<td>1110 ± 462</td>
<td>96 ± 27</td>
</tr>
<tr>
<td>3</td>
<td>DBA/2J</td>
<td>10203 ± 4415</td>
<td>275 ± 126</td>
</tr>
<tr>
<td></td>
<td>C57B1/6</td>
<td>6891 ± 265</td>
<td>1126 ± 609</td>
</tr>
<tr>
<td></td>
<td>B_6.D_2.F_1</td>
<td>4896 ± 4577</td>
<td>265 ± 93</td>
</tr>
</tbody>
</table>
Table 2: Immune response of CBA/J mice to R.Fab' on C57B1/6, DBA/2J or CBA/J spleen cells. Results are expressed as PFC per spleen ± S.E.M. \( P = <0.01 \) according to Student's t test (combined allogeneic vs. combined syngeneic). Each number represents the average of 3 mice.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>R.Fab' coated cells injected</th>
<th>Anti R.Fab' PFC/spleen ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57B1/6</td>
<td>36395 ± 1429</td>
</tr>
<tr>
<td></td>
<td>CBA/J</td>
<td>16198 ± 1119</td>
</tr>
<tr>
<td>2</td>
<td>DBA/2J</td>
<td>33395 ± 8976</td>
</tr>
<tr>
<td></td>
<td>CBA/J</td>
<td>15754 ± 193</td>
</tr>
</tbody>
</table>
The possibility that these differences in response to R.Fab' fragments might simply be an effect of association of these molecules with either syngeneic or allogeneic cells rather than being a result of the Fab' fragments being attached to the cell surfaces was also examined. Fab' fragments with no specificity for mouse cell surface antigens were prepared and mixed with either syngeneic or allogeneic cells at concentrations equivalent to those used with labelled cells. The time course of the responses of C57Bl/6J mice immunized with either C57Bl/6J or DBA irradiated spleen lymphocytes mixed with 10 μg Fab' was followed (Table 3). It can be seen that no significant differences occurred between the 2 groups of immunized mice.

Further studies were carried out to determine the effect of in vitro culture of immune spleen lymphocytes in the presence of varying concentrations of R.Fab'. Over a range of 2.0 to 30.0 μg of Fab' per culture, immune lymphocytes responded specifically to the R.Fab' fragments. A dose level of 10 μg Fab' per culture was established since it appeared to be close to optimal levels and was equivalent to the in vivo stimulus. A series of experiments were performed using C57Bl/6 or CBA mice as recipients of labelled syngeneic, allogeneic or F₁ lymphocytes. The PFC per 10⁷ cells was determined at the time of sacrifice (5 days after the final immunization) and aliquots of spleen cells were subsequently cultured with 10 μg R.Fab' for 4 days, harvested and assayed for PFC with R.Fab' coated SRBC. The results are summarized in Table 4. It can be seen that while the PFC in recipients of either syngeneic or F₁ labelled cells was depressed at the time of sacrifice in comparison to recipients of allogeneic labelled cells, the PFC to R.Fab' fragments in the former group, after in vitro culture was equal to or greater than those observed in the latter group.
Table 3: Immune response of C57B1/6 mice to 10 μg R.Fab' mixed with either C57B1/6 or DBA/2J irradiated splenic lymphocytes (R.Fab' not specific for mouse cells). Results are expressed as PFC per 10⁷ host spleen cells ± S.E.M. Each number represents the average of 2 mice.

<table>
<thead>
<tr>
<th>Injection</th>
<th>Cells Injected (mixed with 10 μg R.Fab')</th>
<th>PFC per 10⁷ responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>2°</td>
<td>DBA</td>
<td>11.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>3°</td>
<td>DBA</td>
<td>65 ± 45</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>80 ± 57</td>
</tr>
<tr>
<td>4°</td>
<td>DBA</td>
<td>177 ± 100</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>325 ± 74</td>
</tr>
<tr>
<td>5°</td>
<td>DBA</td>
<td>136 ± 30</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>162 ± 12</td>
</tr>
<tr>
<td>6°</td>
<td>DBA</td>
<td>18 ± 18</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>42 ± 6</td>
</tr>
</tbody>
</table>
Table 4: Effect of *in vitro* culturing of spleen cells from mice previously immunized with R.Fab' coated lymphocytes of syngeneic or allogeneic origin. Culture medium contained 10 μg R.Fab' (non-specific for mouse cells) per $10^7$ cultured cells. PFC are presented as per $10^7$ recipient spleen cells at the time of sacrifice (Day 0) and after 4 days in culture with 10 μg free R.Fab'.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Recipient</th>
<th>Fab' coated cells injected</th>
<th>PFC/$10^7$ spleen cells DAY 0</th>
<th>PFC/$10^7$ spleen cells DAY 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57</td>
<td>DBA</td>
<td>2280 ± 618</td>
<td>946 ± 116</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>C57</td>
<td>448 ± 204</td>
<td>1329 ± 544</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>B.D.F. 6.2.1</td>
<td>478 ± 203</td>
<td>1674 ± 395</td>
</tr>
<tr>
<td>2</td>
<td>C57</td>
<td>DBA</td>
<td>995 ± 346</td>
<td>233 ± 28</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>C57</td>
<td>575 ± 105</td>
<td>462 ± 137</td>
</tr>
<tr>
<td></td>
<td>C57</td>
<td>B.D.F. 6.2.1</td>
<td>750 ± 662</td>
<td>404 ± 76</td>
</tr>
<tr>
<td>3</td>
<td>CBA</td>
<td>C57</td>
<td>2885 ± 567</td>
<td>438 ± 148</td>
</tr>
<tr>
<td></td>
<td>CBA</td>
<td>CBA</td>
<td>1275 ± 125</td>
<td>642 ± 305</td>
</tr>
<tr>
<td>4</td>
<td>CBA</td>
<td>C57</td>
<td>996 ± 154</td>
<td>233 ± 31</td>
</tr>
<tr>
<td></td>
<td>CBA</td>
<td>CBA</td>
<td>282 ± 2</td>
<td>535 ± 119</td>
</tr>
<tr>
<td>5</td>
<td>CBA</td>
<td>DBA</td>
<td>875 ± 450</td>
<td>248 ± 78</td>
</tr>
<tr>
<td></td>
<td>CBA</td>
<td>CBA</td>
<td>341 ± 73</td>
<td>352 ± 124</td>
</tr>
</tbody>
</table>
In vitro Stimulation of Responder Spleen Cells Measured by $^{3}\text{H}$ Thymidine Uptake.

Increased uptake of $^{3}\text{H}$-thymidine by immune lymphocytes cultured in the presence of appropriate concentrations of antigen is thought to be mainly an indication of T cell responsiveness. For this reason it was thought that comparative data could be obtained for T cells from mice sensitized to R.Fab' on syngeneic vs. allogeneic backgrounds. Under no circumstances in the course of these studies could significant levels of stimulation of immune lymphoid cells cultured in the presence of R.Fab' be achieved, regardless of the means of immunization. Mitogen stimulation of these cells by Concanavalin A or LPS in culture was normal.
DISCUSSION

Considerable evidence exists that indicates immune homeostasis is the result of a balance between responsiveness and suppression, and that non responsiveness to self antigens is the result of active suppression (22-28). Evidence presented by Scibienski et al (14) (i.e. non responsiveness to an antigen linked to a tolerogen) suggests active suppression against the tolerogen, which extends to a new antigen, coupled to that tolerogen. Lee and Sehon (15) using haptenated mouse immunoglobulin, showed that the suppression to the hapten is maintained during adoptive transfer experiments, implying the presence of suppressive cells, whose suppression extends to new determinants coupled to the tolerated antigen.

Humoral autoreactivity has, in the past, been enhanced by the administration of LPS (29) or rabbit anti-mouse thymocyte serum (30-22), the explanation in the latter case, being the removal of suppressive T cells. Although conclusive evidence for the presence of cells which suppress autoreactivity is not presented in this study, the data support such a theory. Rabbit Fab' fragments, when attached to syngeneic or semi syngeneic (F1) cells, generate a decreased humoral immune response, when compared to the response mounted against the same antigen presented on allogeneic cells. This observation appears to hold regardless of the strain combinations used. If non responsiveness to self is maintained by suppressor cells, then, as was the case in Scibienski's work, this non responsiveness includes antigens which are attached to the tolerated antigen (in this case self, cell surface antigens). If, on the other hand, the response toward R.Fab' on syngeneic cells was not suppressed, but the response towards R.Fab' on allogeneic cells was enhanced, due to the presence of alloantigens, one would also expect an enhanced
response to the same antigen on any cells bearing allogeneic antigens. This is not the case, the response towards R.Fab' on semi syngeneic (also semi allogeneic) F₁ cells was as low as that towards R.Fab' on syngeneic cells. Thus, it would appear to be the presence of self antigens which cause the decreased response, rather than the presence of allogeneic antigens which enhance the response. The observation that background anti-SRBC plaques in our assays were never markedly different between experimental groups would indicate that in the conditions used here, no marked allogeneic effect was taking place.

Abrogation of self tolerance in vitro has in the past, been accomplished by incubating immune competent cells with self cells (25, 26), with LPS (31, 32) or by incubation in culture medium alone (33). These findings are consistent with the results obtained in this study: those spleen cells which showed a low anti R.Fab' response on day 0 (Table 4), i.e. spleen cells from those mice receiving R.Fab' on syngeneic cells, after 4 days in vitro incubation with soluble antigen, showed a response equal to or larger than spleen cells from those mice receiving R.Fab' on allogeneic cells. This suggests the presence in immunized animals of potentially reactive cells, but that their development into antibody producing cells is in some way inhibited in vivo.

Suppression of the humoral response against heterologous antigens on the surface of 'self' cells, seems an inviting explanation for the weak immune reactivity seen toward tumour antigens in many instances.

Recent work in animal systems (tumours and virally infected cells) has indicated that potent cell mediated immunity, assayed by the in vitro generation of cytotoxic cells, can be developed against syngeneic cells demonstrating altered antigenicity. Indeed, these cytotoxic cells are only able to deliver the killing signal to those modified target cells
sharing at least one of the major histocompatibility antigens (5, 11, 8). These observations would appear to be in contrast to those reported here. However, it should be pointed out that while marked cytotoxicity can be measured in mice against their syngeneic tumors, this response appears to be under rapidly developing stringent and specific suppression mediated by T cells (34).

Whether or not the lowered humoral response seen in animals immunized with an antigen linked to syngeneic cells is a manifestation of specific suppression of self or self-associated cell surface markers remains to be clarified. It is, however, clear that the association of an antigen with syngeneic, as opposed to allogeneic cell surface antigens does have a significant effect on the humoral response to that antigen.
BIBLIOGRAPHY


