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IMMUNE RESPONSE TO IMMUNIZATION VIA THE ANTERIOR CHAMBER OF THE EYE

II. An Analysis of F_1 Lymphocyte-Induced Immune Deviation

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Exposure to alloantigen via the anterior chamber of the eye elicits a transient suppression of cellular immunity, whereas humoral immunity is preserved—i.e. F_1 LI-ID. The majority of lymphoid cells inoculated into the anterior chamber are retained within the posterior segment of the eye. The latter serves as a depot of alloantigen, allowing the chronic egress of small numbers of cells into the vascular tree. The persistence of this antigen depot is essential to the development of F_1 LI-ID. Since there is a preferential distribution of cells that migrate from the eye to the spleen, the functional integrity of the latter is also necessary to elicit F_1 LI-ID. It is concluded that an anatomically intact spleen, i.v. presentation of antigen, and persistence of antigen within the eye are all important to the elicitation of this phenomenon.

In previous communications (1–4) we have emphasized the unique immunologic features of the anterior chamber of the eye—namely, the absence of demonstrable lymphatic drainage, the existence of partial immunologic privilege for alien tissue grafts (despite an intact immunologic reflex arc), the failure of refractoriness to appear after resolution of the acute phase of local lymphocyte transfer reactions, and the existence of F_1 lymphocyte induced-immune deviation (F_1 LI-ID).[3] The latter phenomenon is observed after the anterior chamber injection of F_1 hybrid lymphocytes in parental rats. A transient systemic suppression of cell-mediated immunity develops despite a vigorous humoral response. The expression of F_1 LI-ID depends on the immunogenic properties of motile, semi-allogeneic cells; additionally, it possesses immunologic specificity (4).

This manuscript examines the mechanism of F_1 LI-ID, as well as the migratory pattern of lymphoid cells after inoculation into the anterior chamber of the eye. We found that the suppression of cell-mediated immunity after anterior chamber sensitization is dependent upon antigenic persistence and the continued function of the spleen.

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[3] Abbreviations used in this paper: F_1 LI-ID, F_1 lymphocyte-induced-immune deviation; FI, Fischer; DA, Dark Agouti; HBSS, Hanks' balanced salt solution; NRS, normal rat serum; NRbS, normal rabbit serum; GVH, graft-*vs*-host; HVG, host-*vs*-graft; fp, footpad; MST, median survival time.

MATERIALS AND METHODS

Experimental rats. The animals used in the studies to be described belonged to domestically maintained sublines of the allogeneic Fischer (FI-AgB[1]), DA (AgB[4]) and their F_1 hybrids.

Lymph node cell suspensions, orthotopic skin grafts, and anterior chamber inoculations. These techniques have been previously described in detail (4).

Hemagglutinating antibody titers. Sera were tested by the method of Gorer and Mikulska (5), with slight modifications. One milliliter of heparinized FI blood was obtained by cardiac puncture and the red blood cells (RBC) were washed five times in 40 ml of phosphate-buffered saline (PBS), pH 7.4. Finally, the RBC were suspended as a 2% solution of 3.8% sodium citrate (PBS). Serial 2-fold dilutions of 25 μl of experimental sera were made in microtiter hemagglutination plates (Cooke Engineering Co., Alexandria, Va.) with a steel microdilutor, in 25% normal rat sera (NRS-PBS). Twenty-five microliters of a 2% dextran- (1:1:, high:low m.w. dextran) glucose (2 g) solution and 25 μl of the 2% RBC solution were then added to each well. After incubation at 37°C, for 1 hr and at room temperature for 90 min the highest serum dilution that resulted in macroscopic hemagglutination was recorded.

Lymphocytotoxic antibody titers. With Herberman and Oren's technique (6) a suspension of FI lymph node cells was washed twice with 10% decomplemented fetal calf serum and Hanks' balanced salt solution (FCS-HBSS) and adjusted to 20×10^6 cells/ml. One milliliter of the FI lymphocyte suspension was incubated with 200 μCi of ^{51}Cr (as sodium chromate, specific activity > 10 μCi/μg Cr, at a concentration of 2 mCi/ml, Radiochemical Center, Amersham, England) at 37°C for 30 min. The labeled cells were washed twice in 40 ml FCS-HBSS, adjusted to 1×10^6/ml (10% NRS-PBS), and 0.1 ml was added to 0.1 ml of serial 2-fold dilutions of rat serum, in 10 x 75 plastic test tubes (Falcon Plastics, Oxnard, Calif.) After incubation at 37°C, for 30 min, 0.1 ml of 1:4 rabbit serum (NRbS-PBS) was added as the source of C and the incubation was continued for an additional 30 min. Two milliliters of cold FCS-HBSS were then added to each tube, the cells were sedimented at 500 × G for 10 min, and 1 ml of the supernatant fluid of each tube was counted for released radioactivity in a Baird-Atomic gamma scintillation counter. The titer of serum was defined as the serum dilution producing ^{51}Cr release twice that of the NRS control, which was usually 15% of the maximum ^{51}Cr release by freezing and thawing the cells three times.

Preparation of ^{51}Cr-labeled lymph node cells. The method used was modified from Heslop and Hardy (7). The superficial and deep cervical and axillary, mesenteric, aortic, and iliac lymph nodes were collected into cold (4°C) HBSS with 1% heat-inactivated normal calf serum and 1% penicillin-streptomycin (50 units, 50 μg/ml, respectively, Grand Island Biological Co., Grand Island, N. Y.). Lymph nodes were pressed through a stainless steel sieve into HBSS. The suspension was then washed once in HBSS and resuspended in cold HBSS at 50×10^6 cells/ml and incubated for 30 min at 37°C with 50 μCi/ml of ^{51}Cr in sterile isotonic saline (Radiochemical Centre, Amersham). The suspension was subsequently washed three times in cold HBSS and suspended at approximately 4.0×10^8 cells/ml for injection. Viability as determined by exclusion of 1:2000 trypan blue was always 90%. Samples of cell suspension were retained for determination of the total label injected and samples of the supernatent were counted to ensure adequate washing of the labeled cells.

Dosage of ^{51}Cr-labeled lymph node cells. All animals received a standard inoculation of 4×10^6 cells. The anterior chamber inoculum consisted of 10μl at a cell suspension of 4×10^8 cells/ml. For i.v. and footpad (fp) injections serial dilutions with HBSS were made to obtain 4×10^6 cells/0.1 ml. Total radioactivity in the inoculum varied between 5000 and 15,000 cpm.

Measurement of radioactivity. The animals were killed at varying times after injection. Lymph nodes (the same set as described above), spleen, liver, lungs, eyes, foot, and 1 ml of blood were removed, and their radioactivity measured for 10 min in a well-type scintillation counter. After subtraction of the background levels, the radioactivity in a given organ was expressed as a percentage of the total dose recovered.

RESULTS

Peripheral distribution of lymphoid cells inoculated into the anterior chamber of the eye. During our initial studies of lymphocyte transfer reactions within the anterior chamber (2) we observed that the bulk of isogeneic lymphoid cells inoculated into the anterior chamber disappeared within the first 12 to 24 hr. During an immunogenic uveitis induced by the injection of allogeneic lymphocytes a similar pattern was observed, except that 72 to 96 hr later lymphoid cells returned into the anterior chamber and a fulminant iritis developed. To study the migratory path of lymphocytes after injection into the anterior chamber we employed radioactive labeling.

Initially, four million ^{51}Cr-labeled FI lymph node cells were injected either i.v., intracamerally, or into the fp of FI rats. The recipients were killed at either 24 or 96 hr and the radioactive content of their tissues was measured in a gamma well counter. As shown in Table I, the overwhelming majority of lymphocytes inoculated into the anterior chamber were still within the eye at 24 hr. Since very few cells were seen within the anterior chamber by slit lamp examination, it appeared that most of the lymphocytes had migrated posteriorly into the uvea. Although there was a continued small egress of cells from the eye within the next 3 days, it still retained 76% of the radioactive inoculum which could be recovered at 96 hr. Those lymphocytes which left the eye were equally distributed among the spleen, liver, and lymph nodes, with a proportionate increase in the number of cells recovered from each organ system at 96 hr. Although the total number of cells leaving the eye and entering these organs was similar, there was a definite preferential concentration of lymphocytes in the spleen and lymph nodes. Each organ's ability to trap circulating lymphocytes is represented in Figure 1. At both 24 and 96 hr the spleen and peripheral lymphatic tissue proved far more efficient at retaining circulating lymphoid cells than the liver. It should also be noted that only one eye of each animal was injected, and that at no time did the ipsilateral cervical lymph nodes have an increased number of labeled lymphocytes compared to the contralateral nodes.

The migratory pattern of lymphocytes after the i.v. or fp injection of isogeneic lymphocytes differed from that just described for the anterior chamber. After i.v. injection (Table I) most cells were found within the liver and spleen, with fewest number recovered from the lymph nodes. In contrast, the fp injection resulted in a majority of the inoculum being retained

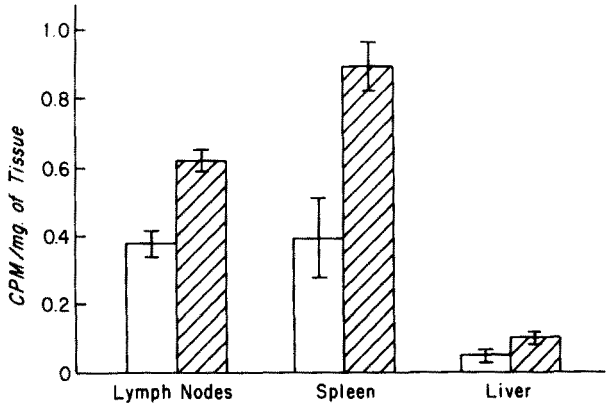

Figure 1. Distribution (expressed as cpm/mg of tissue) of 4×10^6 FI lymphoid cells, at 24 hr (□) and 96 hr (▨), after anterior chamber inoculation in adult FI rats ($n = 6$). Bars represent 1 S.D.

TABLE I

Distribution of ^{51}Cr-labeled isogeneic lymphoid cells after inoculation by different routes[a]

Route	Hr after Injection	Distribution of radioactivity[b, c]						% of Radioactive Inoculum Recovered
		Eye	Foot	Popliteal nodes	Other lymph nodes	Spleen	Liver	
Anterior chamber	24	88.7 ± 4.64	0	0	3.8 ± 1.97	2.8 ± 1.65	4.7 ± 1.16	37.6 ± 24.0
	96	75.9 ± 4.83	0	0	7.9 ± 3.2	5.2 ± 0.998	10.8 ± 2.23	36.8 ± 14.9
Intravenous	24	0	0.1 ± 0.06	0.2 ± 0.15	17.5 ± 3.02	22.0 ± 2.06	60.2 ± 2.96	45.7 ± 9.44
	96	0	0.1 ± 0.08	0.2 ± 0.08	14.8 ± 3.72	22.8 ± 6.68	62.0 ± 7.31	36.3 ± 3.89
Footpad	24	0	89.5 ± 2.65	7.1 ± 1.98	0.8 ± 0.31	0.4 ± 0.16	2.1 ± 0.69	51.2 ± 3.85
	96	0	83.4 ± 7.74	8.1 ± 3.58	3.0 ± 2.64	1.4 ± 0.71	4.2 ± 1.99	32.6 ± 4.78

[a] Four million ^{51}Cr-labeled FI lymph node cells were injected via various routes into panels ($n = 6$) of adult FI rats.

[b] Distribution of radioactivity is expressed as the % ± S.D. of radioactive inoculum recovered. Zero represents a distribution of <0.1%.

[c] In each panel, regardless of the route of inoculation, the distribution of radioactivity was < 2% in the lungs, < 0.1%/ml of blood, and < 0.1% in the uninjected eye or foot.

within the foot. Those cells which escaped from this site of inoculation were subsequently trapped within the ipsilateral popliteal lymph node, with very few cells migrating to the liver, spleen, or other lymphatic tissue. No significant change in migratory pattern was observed between 24 and 96 hr.

A similar series of experiments were then performed with semi-allogeneic (FI × DA) F_1 lymphoid cells inoculated into FI recipients, the same genetic combination used to produce F_1 LI-ID. The results were no different from those obtained with isogeneic lymphocytes (Table II). Consequently, the following conclusions can be drawn regarding the migratory pattern of lymphocytes: 1. The majority of lymphoid cells injected into the anterior chamber remain within the eye for at least 4 days, similar to the local retention of lymphocytes following fp inoculation; 2. Those lymphocytes that migrate from the eye have a peripheral distribution to the spleen, liver, and lymphatic system—a pattern similar to that observed after the i.v. injection of lymphocytes, although more cells enter the liver after i.v. inoculation; 3. Those cells that are able to leave a site with lymphatic drainage, i.e., the fp, are preferentially trapped in the local lymph nodes. It seems that because there is no demonstrable lymphatic drainage from the anterior chamber lymphocytes inoculated into the anterior chamber are predicated to leave it through the vascular tree, probably via the choroidal circulation.

Importance of the spleen in F_1 LI-ID. Since lymphocytes inoculated into the anterior chamber leave the eye by the i.v. route, and since the spleen receives the brunt of antigen administered i.v., the effect of splenectomy on F_1 LI-ID was studied. Three weeks before anterior chamber immunization FI recipients underwent splenectomy. Surprisingly, F_1 LI-ID was abrogated. The enhanced median survival time (MST) of orthotopic (FI × DA)F_1 skin grafts placed 10 days after the anterior chamber inoculation of 10 million (FI × DA)F_1 lymph node cells in spleen intact hosts was abruptly curtailed from 12.0 to 8.3 days in previously splenectomized animals. The latter MST is similar to a first set reaction, 9.1 days, and is significantly different from that seen in spleen-intact hosts (Table III). In

TABLE II

Distribution of ^{51}Cr-labeled semi-allogeneic lymphoid cells after inoculation by different routes[a]

Route	Hr after Injection	Distribution of Radioactivity[b, c]						% of Radioactive Inoculum Recovered
		Eye	Foot	Popliteal nodes	Other lymph nodes	Spleen	Liver	
Anterior chamber	24	87.4 ± 3.19	0	0	4.0 ± 1.67	3.8 ± 1.38	4.6 ± 2.50	63.3 ± 12.83
	96	74.2 ± 6.21	0.2 ± 6.21	0	9.0 ± 4.04	7.2 ± 1.64	9.5 ± 1.68	39.1 ± 14.49
Intravenous	24	0	0	0.1 ± 0.1	9.0 ± 4.37	23.7 ± 9.95	67.2 ± 11.8	47.7 ± 3.50
	96	0	0.4 ± 0.20	0.6 ± 1.13	13.1 ± 9.67	23.3 ± 10.3	62.8 ± 20.36	35.8 ± 8.31
Footpad	24	0	87.8 ± 1.44	8.6 ± 2.15	1.0 ± 0.89	0.7 ± 0.19	1.9 ± 1.72	
	96	0	87.3 ± 8.99	6.4 ± 3.44	2.5 ± 1.42	1.0 ± 0.55	2.8 ± 1.30	

[a] Four million ^{51}Cr-labeled (FI × DA)F_1 lymph node cells were injected via various routes into panels ($n = 6$) of adult FI rats.

[b] Distribution of radioactivity is expressed as the % ± S.D. of radioactive inoculum recovered. Zero represents a distribution of < 0.1%.

[c] In each panel, regardless of the route of inoculation, the distribution of radioactivity was < 2% in the lungs, < 0.1%/ml of blood, and < 0.1% in the uninjected eye or foot.

TABLE III

Effect of splenic function on F_1 LI-ID

Route of Immunization[a]	Days Grafted Postimmunization[b]				
	7	10	14	17	21
Anterior chamber					
Spleen-intact	8.5 ± 1.1 (5)	12.0 ± 1.0[c] (20)	9.4 ± 1.2 (18)		
Splenectomized[d]		8.3 ± 1.1 (15)	9.4 ± 1.2 (8)	9.3 ± 1.1 (8)	7.8 ± 1.0 (5)
Intravenous					
Spleen-intact	7.8 ± 1.1 (8)	8.4 ± 1.1 (10)	8.4 ± 1.2 (5)		
Splenectomized		7.8 ± 1.0 (10)			
Footpad					
Spleen-intact	7.8 ± 1.0 (10)	7.8 ± 1.0 (10)			
Splenectomized		7.7 ± 1.0 (8)			

[a] Ten million (FI × DA)F_1 lymph node cells were injected into panels of adult FI rats by different routes. The MST of orthotopic (FI × DA)F_1 skin grafts, placed on these animals at various intervals postimmunization, was then determined.

[b] The MST, days ± S.D., is recorded under the days postimmunization that the sensitized recipients were grafted orthotopically with (FI × DA)F_1 skin. The number of animals in each experimental panel is in parentheses.

[c] A first set (F_1 × DA)F_1 → FI MST = 9.1 ± 1.1, $n = 15$; a second set MST = 7.8 ± 1.0, $n = 9$. With the Student's t-test, only spleen-intact animals sensitized via the anterior chamber showed a significantly prolonged MST, compared to a first set MST, $p < 0.001$. This effect was abolished by prior splenectomy. The range of MST for the spleen-intact animals was 9 to 18 days, whereas that for the splenectomized panel was 7 to 10 days.

[d] Splenectomized FI rats underwent splenectomy 3 weeks before sensitization with (FI × DA)F_1 lymphoid cells.

contrast, splenectomized recipients immunized by either the i.v. or fp injection of F_1 lymphoid cells did not have significantly altered MST for subsequent orthotopic F_1 skin grafts. Only the development and expression of F_1 LI-ID were closely linked to the presence of the spleen at the time of anterior chamber immunization.

We had previously reported (4) that the intracameral inoculation of semi-allogeneic lymphocytes elicited a vigorous serum hemagglutinating antibody response before the development of F_1 LI-ID. Consequently, the effect of splenectomy on this aspect of the immunologic response was studied. FI rats were splenectomized 3 weeks before the anterior chamber inoculation of 10 million F_1 lymphocytes and bled serially on days 4, 7 and 10. As depicted in Figure 2, there was a marked delay in the hemagglutinin response, with barely detectable levels on days 4 and 7, and reduced titers on day 10. A similar pattern was observed when lymphocytotoxic antibody titers were determined. Spleen-intact recipients had serum titers of 1:8 (n = 7) on day 4, ≥1:64 (n = 7) on day 7, and ≥1:64 (n = 5) on day 10; whereas, splenectomized hosts had serum titers <1:4 (n = 6) on day 4, <1:4 (n = 5) and 1:32 (n = 1) on day 7, and <1:4 (n = 1) and 1:16 (n = 3) on day 10. These results suggested that: 1) the rejection of F_1 orthotopic skin grafts was not related to the generation of lymphocytotoxic antibody, and 2) the delay in appearance of the antibody response in splenectomized hosts implied that F_1 LI-ID might not be abrogated but rather delayed till a more effective humoral response could be generated.

To explore this possibility panels of FI rats were splenectomized 3 weeks before anterior chamber immunization with F_1 lymphoid cells. However, instead of orthotopic skin grafts being placed 10 days after intracameral sensitization, they were placed on days 14, 17, or 21. As can be seen in Table III, the MST of these grafts were not enhanced. It appears that splenectomy before anterior chamber sensitization not only delays the antibody response but alters the qualitative character of the immunologic response such that F_1 LI-ID is completely abrogated.

Persistence of splenic function in F_1 LI-ID. The previous experiments established that presence of the spleen at the time of anterior chamber immunization was essential for the development of F_1 LI-ID. Further insight into the mechanism by which the spleen might interfere with the systemic expression of cell-mediated immunity was obtained by performing splenectomy at specified intervals post-sensitization. FI rats were injected with 10 million (FI × DA)F_1 lymph node cells into the anterior chamber and splenectomized 1 and 4 days later. They were bled on days 4, 7, and 10, and orthotopically grafted with (FI × DA)F_1 skin on day 10. Figure 2 displays the hemagglutinin response, which was barely detectable on days 4 and 7, and markedly suppressed on day 10 in the panel splenectomized 24 hr after anterior chamber inoculation. The MST of grafts on these animals = 7.6 ± 1.1 days, n = 10, which is a second set reaction. Those animals splenectomized 4 days after immunization showed moderately depressed hemagglutinating antibody titers (Fig. 2), with a MST = 9.7 ± 1.2 days, n = 9, which is a first set reaction. These results imply that the continued functioning of the spleen is required for the full development and expression of F_1 LI-ID. During the first 4 days after immunization the spleen is actively modifying the nature of the developing immunologic response since serum antibody titers were higher and the MST of subsequent F_1 skin grafts prolonged in animals who had a spleen for 4 days in contrast to those splenectomized after 1 day. Nevertheless, the full development of F_1 LI-ID requires the presence of a functional spleen for an even longer period of time.

Role of antigenic persistence in F_1 LI-ID. Our studies with ^{51}Cr-labeled lymphoid cells demonstrated that the majority of lymphocytes inoculated into the anterior chamber were retained within the eye, but that there was a continual slow egress of the residual cells. Such a persistent immunogenic stimulus, even though small in amplitude, could significantly modify the immune response generated. The role of antigenic persistence in the development of F_1 LI-ID was studied by the timed enucleation of inoculated eyes after sensitization. Panels of FI rats were inoculated into their left anterior chamber or fp. They were bled on days 4, 7, and 10, and then grafted with (FI × DA)F_1 skin orthotopically on day 10. The animals underwent either enucleation of their left eye or amputation of their inoculated foot at 8, 24, or 96 hr post-immunization. Within 8 hr of anterior chamber sensitization sufficient antigen has been provided to the host to induce a significant hemagglutinating antibody response (Fig. 3); by 24 hr the normal hemagglutinin response was observed. However, the MST of subsequent F_1 skin grafts on these panels = 8.5 ± 1.2 days (n = 7), 9.3 ± 1.2 days (n = 8), and 9.0 ± 1.2 days (n = 8) for animals enucleated at 8, 24, and 96 hr, respectively. These MST represent a first set reaction and do not provide evidence of suppressed cellular

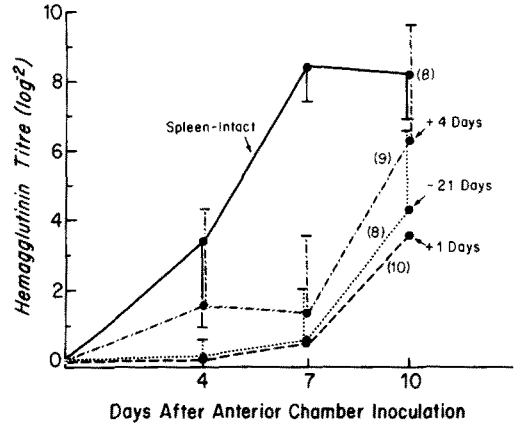

Figure 2. Serum FI anti-DA hemagglutinating antibody titers after anterior chamber inoculation of 10 × 10^6 (FI × DA)F_1 lymph node cells in spleen-intact or splenectomized FI rats. Splenectomy was performed 21 days before (−21) or 1 (+1) or 4 (+4) days after anterior chamber immunization. The number of animals in each panel is enclosed in parentheses.

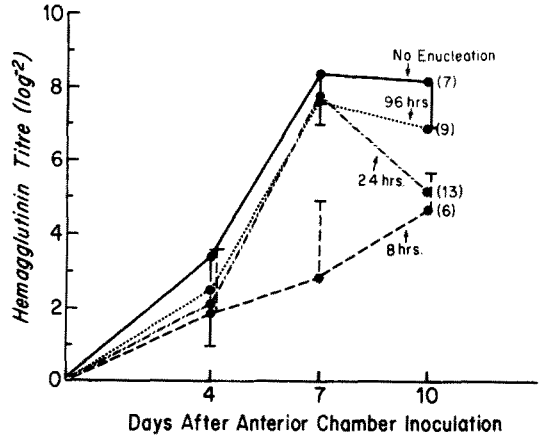

Figure 3. Serum FI anti-DA hemagglutinating antibody titers after anterior chamber inoculation of 10 × 10^6 (FI × DA)F_1 lymphoid cells in FI rats. Injected eyes were enucleated 8, 24, or 96 hr later. The number of animals in each panel is in parentheses.

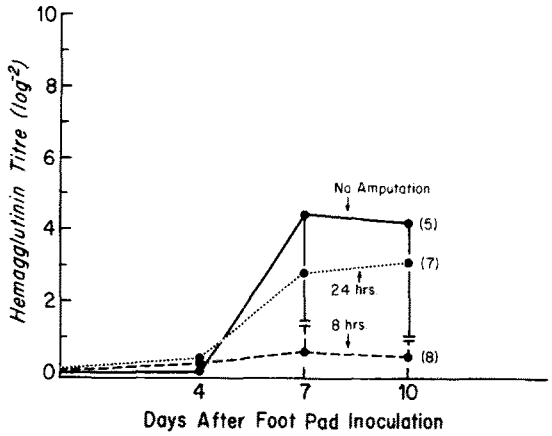

Figure 4. Serum FI anti-DA hemagglutinating antibody titers after footpad inoculation of 10×10^6 (FI × DA)F_1 lymph node cells in FI rats. Injected feet were amputated 8 or 24 hr later. The number of animals in each panel is enclosed in parentheses.

immunity. Interestingly, animals undergoing amputation 8 hr after fp injection had a minimal antibody response (Fig. 4), although significant titers were found in the panel amputated at 24 hr. The MST of subsequent grafts on these animals were consistent with this observation; animals amputated at 8 hr rejected their grafts in a first set fashion, 10.0 ± 1.1 days (n = 8), whereas those in the panel amputated at 24 hr were rejected in a second set fashion, 7.8 ± 1.2 days (n = 7). Thus, it seems that the development of F_1 LI-ID after anterior chamber immunization requires a persistent source of antigen, as well as a functional spleen. In contrast within 24 hr of fp immunization cellular immunity is fully evoked, regardless of splenic function or removal of the primary source of antigen.

DISCUSSION

Aberrant immunologic phenomena frequently have their greatest value in providing an alternative approach to perplexing problems, allowing important insights into the more conventional, but no better understood, events. As such, F_1 LI-ID, the transient suppression of cellular immunity after the presentation of alloantigens to a naive host via the anterior chamber of the eye, has provided important understandings of the interactions between the immune system and the eye. Motile antigenic cells within the eye appear predicated to leave it by the i.v. route, and at a very slow rate. Consequently, the eye serves as a chronic depot of antigen that provides a continual, low amplitude, immunogenic stimulus. Additionally, the immunologic response resulting from the presentation of antigen via the eye is significantly modified by the spleen. The elicitation of F_1 LI-ID requires the continued presence of the spleen. Removal of this organ not only delays and quantitatively alters the humoral response which develops, but qualitatively alters the cellular response exhibited.

The phenomena of F_1 LI-ID *and* immunologic privilege demonstrate that the eye possesses distinctive immunologic characteristics, although the mechanisms by which they are produced may not be the same. The unique immunologic features of this organ seem to revolve about its alymphatic status. This is not to imply that the immunologic reflex arc of the eye is defective. Quite to the contrary, antigen placed within the eye is capable of eliciting an abrupt vigorous immunologic response (3).

In summary, the results presented in this sequence of papers indicate that at least three factors are important to the elicitation of F_1 LI-ID: 1) presentation of intracamerally inoculated antigen to the host via the i.v. route—to the initial exclusion of the peripheral lymphatic system; 2) continued presence of antigen within the eye over at least a 4-day interval after injection; and 3) presence of an intact, functional spleen during the same interval. It is not altogether clear whether there are other factors, which have not been uncovered by these experiments to date, which are equally important. Nonetheless, this constellation of requirements suggests that the spleen provides a relatively unique environment in which antigen presented intracamerally can play a considerable role in modifying the nature of the expected host response. It is not possible on the basis of these findings to decide which of several possible mechanisms might be operative. While we have previously suggested that the experimental design tends to favor the production of enhancing antibodies to account for the prolonged survival of skin allografts (8), we recognize that the data equally well support the possibility that suppressor T lymphocytes might be generated in the spleen (9). Since an anterior chamber containing antigen and an anatomically intact spleen are both required through the first 4 days after injection, and since allografts applied orthotopically 7 days after injection enjoy no prolongation of survival, we conclude that the putative transient sequestration of antigen-reactive lymphocytes within the spleen is unable to account for the F_1 LI-ID phenomenon.

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