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Adoptive Transfer of Cross-Protection Among Alphaviruses in Mice Requires Allogeneic Stimulation

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Cell-mediated (T-effector cell) immunity is proposed as playing the major role in cross-protection between Sindbis and Semliki Forest viruses, which are alphaviruses that do not elicit cross-neutralizing antibodies. In adoptive transfer experiments, T-cells from spleens of Sindbis virus-immunized mice were found to confer specific cross-protection to Semliki Forest virus upon recipient mice. This cross-protection was observed in the outbred ICR strain of mice and when transfers were made between several combinations of inbred and hybrid strains. Cross-protection was substantially reduced if syngeneic rather than allogeneic cell transfers of one spleen equivalent per mouse were made. The results suggest that allogeneic stimulation (mixed lymphocyte reaction in vivo) is necessary to increase the number of effector cells (donor) in the recipient. This was supported by the observation that blastogenic stimulation of donor cells in vitro by concanavalin A induces cross-protection in syngeneic animals. Conversion of recipient cells to specific effector cells also appears to play a role in protecting mice against Semliki Forest virus. This was concluded from the experiments described above, a time course study, and the results of experiments that involved serial passages of transferred cells across histocompatibility barriers. Thus, we propose that both donor and recipient cells are active in protecting recipient mice against challenge with Semliki Forest virus after adoptive transfer.

It has been reported that infection or active immunization with one member of the alphavirus or flavivirus group often results in a heterologous cross-protection against challenge with other members of the same group (5, 8, 14, 15, 25). Cross-protection was shown to occur even between togaviruses, which showed little cross-reaction by hemagglutination inhibition or complement fixation tests and no cross-neutralization (5).

Several studies have definitively shown that infections with viruses that bud off cell membranes elicit cellular immunity (27). The effectiveness of this immunity in such instances has recently been reviewed (26, 30, 33). In the case of cross-protection among togaviruses, our observations have thus far established a role for cell-mediated immunity, particularly in adoptively immunized mice (24). The passive transfer of spleen cells from donor mice immunized with Sindbis virus protected recipient mice against challenge with Semliki Forest virus (SFV) (24). Furthermore, the passive transfer of cross-protection was found to be transmitted by a T-lymphocyte-enriched population derived from the donor animals. The unique features of this system to study cross-protection are: (i) the recipient mice are given well-defined cell populations; (ii) no detectable intact, infectious virus or defective viral particles are transferred to recipients; (iii) no antibody to Sindbis virus or SFV can be detected in the recipient; and (iv) protection develops several days after transfer of cells.

In the present report, we show that adoptive transfer of T-cells exhibits immunological specificity and confers cross-protection best when such transfer is made allogeneically or when the donor cells are stimulated with a mitogen before transfer. The protection develops in the recipient within a time course that is consistent with a mechanism for donor cell proliferation and specific sensitization of recipient cells. The hypothesis that sensitization of recipient cells occurs is also supported from serial passage experiments across histocompatibility barriers.

MATERIALS AND METHODS

Animals. A/He, AKR, BALB/c, CBA, C3H/Anf, C57BL/6, DBA/2, 101, ICR/Ha, and CD2F$_1$ (BALB/c × DBA/2) mice were obtained from Cumberland View Farms, Clinton, Tenn. A/J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Donor mice were 5 weeks old at the time of immuni-

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zation, and recipient mice were 5 weeks old at the time of adoptive transfer and 6 weeks old at the time of challenge.

Viruses. A description of the heat-resistant (HR) strain of Sindbis virus, together with the methods of its cultivation and titration in chicken embryo cell culture, has been reported previously (2, 4, 29). The stock Sindbis virus suspension contained 10^9 plaque-forming units (PFU) per ml. The SFV was obtained from W. P. Allen and contained $10^{9.8}$ PFU/ml. This was equivalent to between 10^6 and 10^7 50% intraperitoneal lethal doses ($MIPLD_{50}$). Stocks of vesicular stomatitis virus (Indiana strain) and vaccinia virus, which were used as immunization and challenge controls, were grown in chicken embryo cell cultures and titrated to $10^{8.1}$ and $10^{8.3}$ PFU/ml, respectively. To assure consistent results, uniform concentrations of immunizing virus (10^7 PFU/mouse, given in each of two intraperitoneal injections 10 days apart) and challenge virus (10^3 50% intraperitoneal lethal doses; equivalent to $10^{5.3}$ PFU of SFV per mouse or 10^8 PFU of vaccinia virus per mouse) were administered.

Spleen cell suspensions. Spleen cells were obtained from virus-immunized (Sindbis virus or control viruses) or sham-immunized (diluent plus spent tissue culture media) donor mice (24). Briefly, spleens from each donor group were pressed through a stainless steel grid into Hanks balanced salt solution (HBSS), aspirated thoroughly through a 16-gauge needle, and then filtered through sterile cheesecloth. The resulting cell suspensions were washed three times in HBSS and then adjusted to the desired concentration for injection into recipient mice or for treatment in vitro before intraperitoneal injection into recipients as described below.

Purification of lymphocyte populations. To deplete T- or B-cells, spleen cells were treated with either rabbit anti-mouse thymocyte serum (ATS) (Microbiological Associates, Bethesda, Md.) or rabbit anti-mouse gamma globulin (Cappel Laboratories, Downington, Pa.). Spleen cells (10^7/ml in HBSS) were treated for 1 h at 4°C with a 1:15 dilution of either antiserum in the presence of 10% guinea pig complement (Flow Laboratories, Bethesda, Md.). Cells were then washed once with HBSS, incubated for an additional 45 min at 37°C, washed again, and then adjusted to a standard concentration of viable cells (determined by trypan blue dye exclusion) for injection. Cells remaining after treatment with anti-mouse gamma globulin and complement resulted in a population of T-cells that was greater than 95% sensitive to ATS and complement. Likewise, cells remaining after treatment with ATS were greater than 95% sensitive to anti-mouse gamma globulin and complement.

To obtain an enriched T-cell population by an alternative method, spleen cell suspensions were fractionated on nylon fiber columns by the method of Julius et al. (18). The method consisted of a preliminary filtration through glass wool to remove dead cells and adherent cells, followed by incubation of the glass wool-nonadherent cells on nylon fibers for 45 min at 37°C. A highly purified T-cell population (greater than 95% sensitive to antithymocyte serum and complement cytotoxicity as determined by trypan blue dye exclusion) was then eluted from the column with HBSS.

Treatment of spleen cells with ConA. It has been shown by other investigators that use of the mitogen concanavalin A (ConA) can amplify antiviral cellular immune responses by increasing the numbers of sensitized lymphocytes both in vivo and in vitro (16, 31). Therefore, such mitogenic stimulation with ConA was used to induce proliferation of donor cells in the recipient. For those experiments, spleen cells from Sindbis virus-immunized or unimmunized donor mice were prepared as described previously and then incubated for 24 h in medium 199 containing 5% fetal calf serum and 1 μg of ConA (Pharmacia, Uppsala, Sweden) per ml. Cells were then harvested, washed three times with HBSS, and adjusted to $10^{7.4}$ viable cells per ml for injection into recipients.

Statistical treatment of data. Survival patterns of each experimental group were compared and statistically analyzed by using the conversational program EXPVAL, provided by Steven Vas (Toronto Western Hospital, Toronto, Ontario, Canada). The EXPVAL program applies an analysis of variance by negative exponential transformations and t tests (22). A statistical transformation is a method of censoring data based upon a mathematical function of observed survival time. This program employs a transformation assuming that one-half of the surviving animals in each group die on the day after the last day of observation; the other half is assumed to survive indefinitely. Statistically, this transformation represents an assumption of the worst possible case.

RESULTS

Passive cross-protection in ICR mice. To confirm previous observations of the role of cell-mediated immunity in cross-protection between Sindbis virus and SFV (24), a fundamental adoptive transfer experiment was analyzed. The analysis included calculations of the average survival time and a statistical analysis of survival patterns. In this preliminary experiment, spleen cells from Sindbis virus-immunized, ICR random-bred mice were treated in vitro with cytotoxic antisera and complement to deplete the T- or B-cell population and then transferred to recipient animals which were challenged intraperitoneally 6 days later with SFV. Additionally, some recipient groups received undepleted spleen cells processed in parallel with groups treated with the cytotoxic antisera. The results of this cell transfer procedure can be seen in Table 1. Here, $10^{7.4}$ spleen cells, the maximum number obtainable per donor mouse after treatment with cytotoxic antisera, from either Sindbis virus-immunized or unimmunized donor groups, were transferred to recipients. An increased survival could be noted among those groups receiving cells from Sindbis virus-immunized donors, as long as T-cells were present (Table 1, groups 1 and 5). Unprotected groups

(groups 2, 3, 4, and 6) included a variety of controls, all of which consistently showed only a low level of survival in this strain of mice. These controls included mice which received cells from unimmunized animals or no cells at all as well as those receiving Sindbis virus-immune spleen cells treated with ATS. Actively immunized controls (group 7; these mice were immunized with Sindbis virus and challenged with SFV) show the extent of cross-protection after active rather than adoptive immunization. Additional controls (data not shown) established the immunological specificity of cross-protection after both active and adoptive immunizations. These controls included donor mice immunized with vesicular stomatitis virus, whose spleen cells provided no protection against SFV challenge, and recipient mice which were unprotected against challenge with vaccinia virus or vesicular stomatitis virus after a transfer of spleen cells or T-cells from Sindbis virus-immunized donors.

Two general conclusions were drawn from the data in Table 1. First, passive transfer of spleen cells from Sindbis virus-immunized mice confers significant cross-protection against SFV challenge; this was demonstrated by increased survival compared with a variety of controls. Second, it is the T-cell-enriched population of the spleen, not the B-cell-enriched population, which accounts for this protection.

Allogeneic reaction. It has been reported that the immune response to T-cell-dependent antigens can be enhanced as a result of a mixed lymphocyte reaction (19, 23, 28). This allogeneic reaction has been shown to be T-cell dependent and mediated by a factor released by activated allogeneic T-cells (1, 10, 11). The experiments shown in Table 1 made use of ICR mice, an outbred Swiss strain in which the histocompatibility loci were assumed to be non-homogeneous. If, in contrast, syngeneic inbred strains were selected in which allogeneic stimulation of transferred lymphocytes was absent, this might be expected to affect the level of cross-protection. Table 2 shows that the transfer of a T-cell-enriched population (obtained as the effluent of glass wool and nylon fiber columns) from Sindbis virus-immunized donors usually did not confer significant protection to recipients which shared the same major histocompatibility antigens (Table 2; compare groups 1 and 2, 4 and 5, 8 and 9). That is, cell-mediated cross-protection does not appear to occur in the absence of allogeneic stimulation of the transferred cells. This was true in all of the syngeneic inbred strains tested, with the exception of the AKR strain (for groups 1 and 2, $P = 0.01$; this is accepted as significant protection).

If, on the other hand, transfers were made between inbred strains differing at the major histocompatibility complex, cross-protection was again evident (Table 2, groups 3 and 7). Note that only those recipients of Sindbis virus-immune spleen cells passed across H-2 barriers showed substantially enhanced survival over control groups receiving cells from unimmunized donor mice.

Table 3 shows that total (undepleted) spleen cells rather than a T-cell-enriched population

TABLE 1. *Adoptive cross-protection against SFV challenge in ICR mice*[a]

Group	Donor strain[b]	Spleen cells[c] transferred	Sindbis virus immunization	No. of survivors/no. in group	% Survivors	Avg survival time (days)[d]	P
1	ICR	T-cells	+	20/25	80	11.0	<0.0005
2	ICR	T-cells	−	3/25	12	7.5	
3	ICR	B-cells	+	4/25	16	7.5	<0.35
4	ICR	B-cells	−	5/25	20	7.2	
5	ICR	Undepleted	+	17/25	68	10.2	<0.005
6	ICR	Undepleted	−	5/25	20	7.8	
7	None[e]	None	+	21/25	84	11.2	<0.0005
8	None	None	−	3/25	12	6.6	

[a] Recipient mice were challenged with $10^{5.3}$ PFU (10^3 50% intraperitoneal lethal doses) intraperitoneally at 6 days after cell transfer.

[b] Spleen cell donors were immunized by two intraperitoneal injections of 10^7 PFU of Sindbis virus at 10 and 20 days before cell transfer. Unimmunized controls received only spent tissue culture medium and diluent.

[c] T-cells were obtained by depletion of B-cells with anti-mouse gamma globulin and complement; B-cells were obtained by depletion with ATS and complement. Recipients were given $10^{7.4}$ viable cells per mouse intraperitoneally.

[d] Average survival time is a calculation of the average day of death assuming that one-half of the survivors die on the day after the last day of observation and the others survive indefinitely (22).

[e] Actively immunized controls, immunized with Sindbis virus and challenged with SFV.

TABLE 2. *Modulation of cross-protection by the major histocompatibility complex*

Group	Donor strain	Spleen cells[a] transferred	Sindbis virus immunization	Recipient strain	No. of survivors/no. in group	% Survivors	Avg survival time (days)	P
1	AKR (k)[b]	T-cells[c]	+	AKR (k)	6/25	24	9.1	<0.01
2	AKR (k)	T-cells	−	AKR (k)	2/25	8	8.2	
3	AKR (k)	T-cells	+	A/He (a)	14/25	56	9.8	<0.0005
4	A/He (a)	T-cells	+	A/He (a)	9/50	18	6.9	<0.45
5	A/He (a)	T-cells	−	A/He (a)	0/25	0	7.0	<0.05
6	None	None	−	A/He (a)	2/15	13	5.8	
7	A/He (a)	T-cells	+	101 (k)	16/25	64	10.2	<0.0005
8	101 (k)	T-cells	+	101 (k)	4/25	16	7.1	<0.4
9	101 (k)	T-cells	−	101 (k)	7/25	28	8.0	

[a] T-cells were obtained from the effluent of glass wool and nylon fiber columns (27).
[b] Letters in parenthesis refer to H-2 haplotypes.
[c] Refer to Table 1, footnote *c* for techniques.

TABLE 3. *Adoptive transfer of cross-protection across H-2 barriers using undepleted spleen cell suspensions*[a]

Group	Donor strain	Sindbis virus immunization	Recipient strain	No. of survivors/no. in group	% Survivors	Avg Survival time (days)	P
1	C57 (b)[b]	+	C3H (k)	10/20	50	8.3	<0.0005
2	C57 (b)	−	C3H (k)	1/16	6	5.8	
3	BALB/c (d)	+	C3H (k)	6/18	33	6.8	<0.01
4	BALB/c (d)	−	C3H (k)	3/18	16	5.5	
5	C3H (k)	+	C3H (k)	3/14	21	7.3	<0.3
6	C3H (k)	−	C3H (k)	2/12	16	6.8	
7	C3H (k)	+	BALB/c (d)	9/12	75	10.6	<0.0005
8	C3H (k)	−	BALB/c (d)	0/12	0	5.6	
9	C3H (k)	+	C57 (b)	10/18	55	8.7	<0.025
10	C3H (k)	−	C57 (b)	4/14	28	6.5	

[a] Refer to Table 1 footnotes for experimental techniques.
[b] Letters in parentheses are H-2 haplotypes.

may be used to transmit cross-protection across histocompatibility barriers. Again, protection of recipient mice is strongest if allogeneic, rather than syngeneic, transfers are made. For example, when C3H donor cells were transferred to C3H recipients, there was no significant increase in survival compared with controls (Table 3, groups 5 and 6; $P = 0.3$). However, these same C3H donor cells (taken from the same pooled suspension) were fully capable of protecting recipients of the BALB/c strain (groups 7 and 8) or the C57BL/6 strain (groups 8 and 9) against challenge. The failure to transfer cross-protection syngeneically does not appear to be the result of poor immunization in the donor. The protection of C3H recipients by Sindbis virus-immune C57 (group 1) or BALB/c (group 3) donor cells indicates that there is no immunological deficiency preventing the protection of C3H recipients, but rather that allogeneic stimulation of the transferred cells, which probably expands the effector cell population (23), is necessary for protection to be detectable.

Time course of cross-protection. To determine the most effective time of challenge after adoptive immunization (the time at which cross-protection, if present, would be most apparent), as well as to determine the duration of the adoptive immunity, an experiment was designed to document the time course of cross-protection in recipient mice. Figure 1 illustrates the time course of protection against SFV challenge after transfer of either syngeneic or allogeneic spleen cells from Sindbis virus-immunized donor mice. Mice challenged 2 days after either syngeneic or allogeneic cell transfer failed to show increased survival, whereas protection was evident among identical groups challenged 4 or 6 days after transfer. The protection still evident after 14 days, when donor cells are presumably rejected (3), suggests a possible role for recruitment and sensitization of recipient cells.

Enhancement of cross-protection by ConA. The possibility was considered that if

protection of the recipient was due to the activity of the donor cells, then survival of recipients might be substantially enhanced by stimulating the proliferation of these donor cells. Stimulation of antiviral activity by T-cells has been shown by other investigators to be substantially increased by in vitro treatment with ConA (16, 31). If donor cells from animals immunized with Sindbis virus were treated in vitro with ConA and then transferred to recipients, no significant enhancement of protection was observed among recipients of allogeneic transfers (Table 4; for groups 1 and 5, $P = 0.1$). Additionally, treatment with ConA allowed recipients of syngeneic transfers a significant increase in survival (for groups 3 and 7, $P = 0.005$). These results suggest that mitogenic stimulation may substitute for allogeneic stimulation in eliciting an expanded population of sensitized donor cells in the recipient This could occur by donor cell proliferation (16, 31) or donor cell activation as suggested by Green et al. (12) for lectin-dependent cell-mediated cytotoxicity or both. The failure of additional mitogenic (ConA) stimulation to substantially increase levels of protection from allogeneic transfers might imply that the stimulation by mixed lymphocyte reaction or ConA cannot be increased beyond a maximal level.

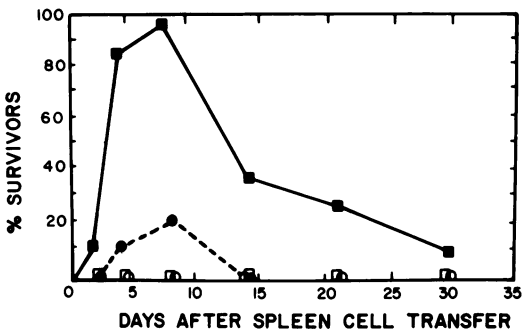

FIG. 1. *Time course of cross-protection in recipient mice. Donor mice of either the C3H or the BALB/c strain were twice immunized with 10^7 PFU of Sindbis virus intraperitoneally or injected without virus as controls. Recipient mice received a single intraperitoneal injection of $10^{7.4}$ viable spleen cells and were challenged at various times thereafter with $10^{5.3}$ PFU (10^3 50% intraperitoneal lethal doses) of SFV. The percentage of survivors was determined at the end of 10 days after challenge. Recipient BALB/c mice were injected with splenocytes from Sindbis virus HR-immune C3H (■), Sindbis virus HR-immune BALB/c (●), nonimmune C3H (□), or nonimmune BALB/c (○).*

Serial passages. To investigate the possibility that a recruitment of specifically sensitized recipient cells may take place after allogeneic stimulation, an experiment was designed to adoptively transfer from one inbred strain into a second and then into the original or second before challenge. Table 5 shows that only recipients of allogeneic transfers show significant differences compared with controls. However, if, instead of challenging these first recipients, spleen cells from these animals were passaged to a second group of syngeneic or allogeneic recipients, a very different pattern emerged. One would expect that if protection of the recipient were solely the result of the activity of stimulated donor cells, a second passage of these cells back into the original donor strain should provide little protection. On the other hand, if only recruited recipient cells were responsible for protection, passage of these cells into syngeneic second recipients should stimulate no additional recruitment and elicit no protection (assuming here that mixed lymphocyte reaction is required as a stimulus for recruitment). As the data indicate, protection was seen in both syngeneic and allogeneic second passage recipients, suggesting that both donor cell proliferation and recipient cell recruitment may play a role in

TABLE 4. *Restoration of syngeneic cross-protection by in vitro stimulation with ConA of transferred spleen cells from mice immunized with Sindbis virus*[a]

Group	Donor strain	Sindbis virus immunization	Concn of ConA (μg/ml)[b]	Recipient strain	No. of survivors/ no. in group	% Survivors	Avg survival time (days)	P		
1	C3H	+	0	BALB/c	8/22	36	8.2			
2	C3H	−	0	BALB/c	3/24	12	6.1	<0.025		
3	BALB/c	+	0	BALB/c	1/22	4	5.8			<0.1
4	BALB/c	−	0	BALB/c	0/22	0	5.1	<0.2		
5	C3H	+	1	BALB/c	12/22	55	10.5		<0.005	
6	C3H	−	1	BALB/c	0/22	0	5.1	<0.005		
7	BALB/c	+	1	BALB/c	11/25	44	9.1			
8	BALB/c	−	1	BALB/c	1/22	4	5.9	<0.005		

[a] Refer to Table 1 for experimental techniques.

[b] ConA-treated spleen cells were incubated for 24 h with 1 μg of ConA per ml in medium 199. Untreated spleen cells were incubated in parallel at 37°C in a 5% CO_2 atmosphere (10^7 cells per ml).

TABLE 5. *Serial passages of spleen cells across histocompatibility barriers*

Donor strain	Sindbis virus immunization	First[a] recipient	Second[b] recipient	No. of survivors/ no. in group	% Survivors	Avg survival time (days)	P
C3H (k)	+	BALB/c (d)	None	7/16	44	8.8	<0.0005
C3H	−	BALB/c	None	1/17	6	5.3	
C3H (k)	+	C3H (k)	None	0/17	0	5.2	<0.45
C3H	−	C3H	None	0/15	0	5.3	
BALB/c (d)	+	BALB/c (d)	None	1/18	5	5.5	<0.2
BALB/c	−	BALB/c	None	0/15	0	5.1	
BALB/c (d)	+	C3H (k)	None	9/18	50	8.5	<0.0005
BALB/c	−	C3H	None	0/15	0	5.4	
C3H (k)	+	BALB/c (d)	BALB/c (d)	6/16	37	7.2	<0.01
C3H	−	BALB/c	BALB/c	1/17	6	5.5	
C3H (k)	+	BALB/c (d)	C3H (k)	8/16	50	8.3	<0.005
C3H	−	BALB/c	C3H	2/16	12	5.7	
BALB/c (d)	+	C3H (k)	BALB/c (d)	11/14	78	10.5	<0.0005
BALB/c	−	C3H	BALB/c	0/12	0	6.0	
BALB/c (d)	+	C3H (k)	C3H (k)	9/12	75	9.4	<0.025
BALB/c	−	C3H	C3H	4/17	23	6.5	

[a] First recipients were injected intraperitoneally with $10^{7.4}$ viable spleen cells from either Sindbis virus-immunized or unimmunized donor mice.

[b] Second recipients were injected intraperitoneally with $10^{7.4}$ viable spleen cells from the group of first recipients in the preceding column.

protecting the recipient animal against challenge.

DISCUSSION

The results of these experiments strongly substantiate the importance of the cellular immune response in cross-protection between Sindbis virus and SFV (24). It was shown that the T-cell population of the spleen could transfer cross-protection and that this immunologically specific protection was optimal when the transferred cells were stimulated to proliferate in the recipient. Stimulation of these cells by adoptive transfer into recipients differing at the H-2 complex and the consequent enhancement of cross-protection are consistent with a mixed lymphocyte reaction occurring in vivo, which can result in enhanced cytotoxicity (32). To our knowledge, this is the first time such an effect has been described for viruses, although allogeneic effects are known to enhance the immune response to a variety of antigens (19, 23, 28). Similarly, factors released from allogenically stimulated T-cells have themselves been shown to enhance the immune response to a number of antigens (1, 10, 11). This suggests the possibility that such factors (lymphokines, such as transfer factor [21]) might be active in converting naive cells into specifically sensitized, immunologically active cells in the recipient animal (6, 7). This recruitment of a protective immunity in the recipient would explain the persistence of cross-protection for up to 30 days after transfer of immune spleen cells, long after the time required for donor cell rejection by the recipient (3). Recruitment of recipient cells to sustain immunity would also be consistent with the results of the serial passage experiment (Table 5). In this experiment, mice having the haplotype of either the original donors or of the first passage recipients were equally protected after consecutive transfers of spleen cells from adoptively immunized mice.

Based on these observations and our previous report (24), we propose that both a proliferation of donor cells and specific sensitization of lymphoid cells by cell contact or lymphokines in the recipient animals are required for optimum cross-protection against the challenge virus over the time course studied. The proliferation of donor and recipient cells and the recruitment of recipient cells would be a consequence of a mixed lymphocyte reaction in an allogeneic host. As expected, this effect would follow transfers made between inbred strains differing at the H-2 complex and between individuals of the outbred ICR strain which is of heterogeneous H-2 composition.

To support this hypothesis, we have demonstrated (Peck, Brown, and Wust, manuscript in preparation) cross-cytotoxicity by lymphocytes from adoptively immunized mice in vitro in the complete absence of either homologous or heterologous antibodies (neutralizing or hemagglutination inhibition). Furthermore, in the in vitro assays, we observed an H-2 restriction in that effector and target cells must be of the same H-2 haplotype, which is consistent with reports

elsewhere with other virus systems (9, 33, 34). We will use H-2 restriction in in vitro cytotoxicity assays, together with appropriate adoptive transfer experiments, to obtain definitive evidence on the relative roles of donor and recipient cells in cross-protection.

That recruitment of recipient cells in our system can occur is supported by the comprehensive model of antigen specific sensitization of T-lymphocyes as a result of interaction with immunogen-reactive allogeneic stimulator cells (6, 7). According to this model, uncommitted "effector" cells are specifically sensitized to chemically modified antigens as a result of contact with allogeneic "initiator" cells. These initiator cells were found to be inactive in effector functions and distinct from the cells they stimulate. In contrast, the recruited lymphocytes were shown to be as capable of mediating the effector functions of cellular immunity as were actively sensitized effector cells.

The focus of this report has been the transfer of protection to adoptively immunized animals, and this appears to principally involve the cellular immune response. However, in actively immunized animals, both humoral and cellular immunity may be operative in cross-protection. The humoral immune response to Sindbis virus has been extensively investigated (17, 20), and an antibody-dependent, complement-mediated cytolysis of SFV-infected target cells by Sindbis virus-immune serum has been reported from our laboratory (20). We have not yet studied the possible role of antibody-dependent cellular cytotoxicity. However, we and others (5, 13) thus far have been unable to correlate the humoral response to Sindbis virus with cross-protection against SFV even in actively immunized animals, although it is generally accepted that antibody plays a major role in homologous protection. Additionally, if antibody is involved in the cross-protection demonstrated after adoptive transfer, immediate cross-protection should be the result, as in most immune serum transfer experiments. We show that cross-protection after adoptive immunization is not observed until at least 4 days after cell transfer. By extrapolation, it seems likely that cell-mediated immunity may play an essential role in protecting even actively immunized animals against heterologous virus challenge. In these animals, the presence of virus-infected cells should provide the stimulus for the proliferation and recruitment of antiviral (or, more correctly, anti-virus-infected target cells) effector cells. One of the chief advantages of the adoptive immunization approach in studying cross-protection is that the contribution of antibody can be minimized.

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