MECHANISMS OF RECOVERY FROM A GENERALIZED VIRAL INFECTION: MOUSEPOX

II. Passive Transfer of Recovery Mechanisms with Immune Lymphoid Cells

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Evidence presented in a previous paper (1) suggested that cell-mediated immunity (CMI)[1] was essential for the process of recovery from mousepox. However, the nature of the antiviral mechanisms involved in this process have not been defined.

Recently, Mackaness has demonstrated that in mice acquired resistance to an intracellular bacterial parasite, *Listeria monocytogenes*, can be transferred with spleen cells from immune donors (2). In this model there is sound evidence that CMI plays an essential part in the generation of antibacterial mechanisms, although the ultimate effectors of resistance are macrophages with enhanced but nonspecific microbicidal activity. In view of these findings, it appeared that passive transfer experiments should provide further information concerning the role of CMI-dependent mechanisms in recovery from mousepox.

Materials and Methods

General.—Details of the stocks of virulent (Moscow strain) and avirulent (Hampstead egg strain) ectromelia virus, and of rabbit anti-mouse thymocyte serum (ATS), and assays for virus plaque-forming units (PFU), neutralizing antibody, interferon, and hypersensitivity to viral antigens were given in a previous paper (1).

Animals.—C57BL mice were employed in most experiments; CBA mice were used only where indicated in the text. Mice of the same age (6–10 wk), sex, and strain were employed in individual experiments.

Spleen Cell Suspensions.—Spleens were cut into pieces and pressed through stainless steel sieves into ice-cold Eagle's minimal essential medium containing 2% fetal calf serum. After dissociation of clumps by pipetting, the suspensions were filtered once through tightly packed 3 × 0.5 cm cotton-wool columns (2), washed three times in Puck's A saline, and suspended for injection in Puck's A saline containing 5 IU of heparin/ml. Viability of the final suspensions was assessed by trypan blue (0.5%) exclusion. It was usually between 75 and 90%.

[1] *Abbreviations used in this paper:* ATS, rabbit anti-mouse thymocyte serum; CMI, cell-mediated immunity; NMS, normal mouse serum; NRS, normal rabbit serum; PFU, virus plaque-forming units.

Anti-Ectromelia Serum.—The hyperimmune serum pool has been described in the preceding paper (1). In repeated assays the ectromelia neutralization titer was 1:4000.

Mouse Interferon.—The stock preparation of mouse brain interferon, stimulated by West Nile virus, contained 400 units/ml.

Bacteria.—The strain of *Listeria monocytogenes* used has been described elsewhere (3). It was virulent for the mouse (intravenous LD_{50} approximately 5×10^4 in C57BL mice). Bacteria were grown in tryptic soy broth (Difco Laboratories Inc., Detroit, Mich.); log phase cultures were dispersed by ultrasound and diluted in Hanks' balanced salt solution with 1% fetal calf serum for injection into mice. Plate counts of viable bacteria were performed on tryptic soy agar (Difco). The method for enumeration of viable bacteria in individual mouse spleens has been given previously (3).

Anti-Light Chain Serum.—Rabbit antiserum against the light chains of mouse immunoglobulins was kindly provided by Dr. Noel Warner.

Anti-Theta Ascitic Fluid.—Ascitic fluid obtained from AKR mice immunized with CBA mouse thymocytes was generously provided by Dr. N. A. Mitchison through Professor Gordon Ada.

Guinea Pig Complement.—Lyophylized guinea pig complement (Commonwealth Serum Laboratories, Melbourne, Australia) was reconstituted, absorbed with CBA thymocytes (which removed lytic activity against mouse cells), and stored at − 20°C.

Irradiation.—Mice were placed in small, circular plastic cages on a rotating stage 20 cm from a ^{60}Co source of gamma rays. Dose rate at this distance was 19.8 rads/min. Controls were subjected to a similar confining treatment without being irradiated.

RESULTS

Passive Transfer of Recovery Mechanisms with Immune Spleen Cells.—

General procedure: Donors of immune spleen cells were immunized intravenously with 5×10^5 PFU avirulent ectromelia virus. Cell recipients were infected intravenously with 2×10^5 PFU virulent ectromelia virus 24 hr before cell transfer so that progressive infection was established in liver parenchyma (4) at the time of injection of cells. Since control of infection in the liver is essential for recovery from primary mousepox (5), the impact of immune cells upon the course of infection was quantified by titrating virus in the liver and spleen at intervals after cell transfer. Although the effects of immune cells in recipients were followed for several days in early experiments, the titers of virus in liver and spleen 24 hr after cell transfer (48 hr after infection) were finally chosen as indicators of antiviral activity. This time sequence avoided the participation of the active response of the recipient, since the virus grew unchecked over the 1st 48 hr after injection.

Preliminary results: Immune spleen cells possessed significant antiviral activity if harvested between 4 and 10 days after donor immunization, but most of this activity was lost by day 20. Normal spleen cells were inactive. The amount of avirulent virus in immune spleen cell inocula was highest in 4-day immune cells (4×10^4 PFU/5×10^7 cells) and declined rapidly thereafter, thus immune cell injections made no significant contribution to virus titers in the recipients.

The following series of experiments, in which 6-day immune donors were used, were designed to elucidate the essential antiviral properties of immune cells.

Effects of Intact Immune Cells, Disrupted Immune Cells, or Serum from 6-day Immune Cell Donors.—

Spleen cells were harvested from immune donors and divided into two portions. One portion was treated with ultrasound for 20 sec so that no intact cells remained as determined by microscopic examination. Disrupted or intact viable cells (4×10^7/recipient) were then injected intravenously into separate groups of five preinfected recipients. Another group of five recipients was given an intravenous injection of serum (0.5 ml/mouse) from the immune cell donors, while a control group was left untreated. An additional group of controls was included to determine virus titers at the time of cell transfer; all other mice were sacrificed 24 hr after cell transfer.

TABLE I

Effect of Intact Immune Spleen Cells, Disrupted Immune Spleen Cells, or Serum from Immune Cell Donors on Virus Titers in the Livers and Spleens of Recipients Infected I.V. with 2×10^5 PFU Virulent Ectromelia Virus 24 Hr before Adoptive Immunization*

Treatment‡	Time after treatment			
	0 hr		24 hr	
	Liver	Spleen	Liver	Spleen
Intact immune cells	—	—	2.7 ± 0.2§	1.7 ± 0.6‖
Disrupted immune cells	—	—	6.4 ± 0.2	5.1 ± 1.2
Immune serum	—	—	6.1 ± 0.3	5.5 ± 0.7
Nil	4.6 ± 0.2	4.4 ± 0.2	6.1 ± 0.3	5.2 ± 0.8

* Expressed as mean log PFU per organ ± standard deviations in groups of five mice.
‡ Donors of immune cells and serum were immunized i.v. with 5×10^5 PFU avirulent ectromelia virus 6 days previously. Doses are given in the text.
§ Significantly less than all other liver counts ($P < 0.001$).
‖ Significantly less than all other spleen counts ($P < 0.01$).

Neither disrupted immune cells nor the serum of cell donors possessed antiviral activity (Table I). Therefore, the fall in virus titers in the livers and spleens of recipients of intact immune cells depended upon the cells functioning within the recipients, not upon preformed factors contained within them.

Interferon and Neutralizing Antibody Production in Recipients of Immune Spleen Cells.—

A group of eight preinfected recipients was injected intravenously with viable immune spleen cells (7×10^7/mouse). Control groups, each of eight mice, received 6×10^7 normal spleen cells or nothing. 10 and 24 hr later, subgroups of four mice were sacrificed and their livers and spleens removed for virus titration. Spleens were snap-frozen in liquid N_2 to preserve interferon (6), and after samples from each mouse had been diluted for virus titration, the crude spleen homogenates of each group were pooled and processed for interferon assays. At 24 hr after cell transfer, serum samples were also obtained from individual mice of each group and assayed for neutralizing antibody.

Table II shows that while significant antiviral activity was evident in the livers and spleens of immune cell recipients, neutralizing antibody was un-

TABLE II

Interferon and Neutralizing Antibody Production in Recipients of Immune Spleen Cells*

Treatment	Time after cell transfer								
	0 hr		10 hr			24 hr			
	Virus PFU‡		Virus PFU		Interferon units/spleen	Virus PFU		Interferon units/spleen	Neutralizing antibody
	Liver	Spleen	Liver	Spleen		Liver	Spleen		
Immune cells§	—	—	6.8 ± 0.4	3.2 ± 0.5‖	0	5.4 ± 1.6¶	4.1 ± 0.9‖	0	<1:10
Normal cells	—	—	7.1 ± 0.2	6.0 ± 0.2	9	8.6 ± 0.1	7.7 ± 0.2	14	<1:10
Nil	6.2 ± 0.3	4.6 ± 0.4	6.4 ± 0.4	5.6 ± 0.3	9	8.1 ± 0.4	7.3 ± 0.6	17	<1:10

* Recipients were injected i.v. with 2×10^5 PFU virulent ectromelia virus 24 hr before cell transfer.

‡ Expressed as mean log PFU per organ ± standard deviations in groups of four mice.

§ Harvested from donors immunized i.v. with 5×10^5 PFU avirulent ectromelia virus 6 days previously. Viable cell doses are given in the text.

‖ Significantly less than both control groups ($P < 0.01$).

¶ $P < 0.05$.

detectable in all their individual sera tested at the minimum practicable dilution, and interferon was not present in their spleens. However, splenic interferon was readily detected in mice which did not receive immune spleen cells.

Effects of Passively Administered Hyperimmune Serum and Interferon.— The preceding experiment gave no indication of the amount of antibody which might be required to achieve the degree of antiviral activity exhibited by immune spleen cells, and again showed that virus growth occurred despite a splenic interferon response, as demonstrated previously (1). Therefore the effects of passively administered hyperimmune mouse anti-ectromelia serum and interferon were investigated.

Groups of five preinfected recipients were given intravenous injections of 0.2 ml/mouse of hyperimmune serum or of interferon (80 units). Control groups received either 0.2 ml of normal mouse serum or nothing. Livers and spleens of all recipients were removed for virus titration 24 hr later. Recipients of normal and immune serum were also bled for neutralizing antibody assays at this time.

TABLE III

Effect of Hyperimmune Serum and Interferon on Virus Titers in Recipient‡ Target Organs*

Treatment	Dose i.v./mouse	Time after serum and interferon injection				
		0 hr		24 hr		
		Liver	Spleen	Liver	Spleen	Neutralizing antibody§
Hyperimmune serum	0.2 ml	—	—	5.1 ± 0.4‖	4.6 ± 0.4¶	100 ± 61
Normal serum	0.2 ml	—	—	7.2 ± 0.3	6.5 ± 0.3	<10
Interferon	80 units	—	—	7.2 ± 0.2	6.5 ± 0.3	—
Nil	—	5.4 ± 0.3	4.6 ± 0.3	7.0 ± 0.3	6.2 ± 0.8	—

* Expressed as mean log PFU per liver or spleen ± standard deviations in groups of five mice.

‡ Recipients were injected i.v. with 2×10^5 PFU virulent ectromelia virus 24 hr before serum and interferon injection.

§ Means of reciprocals of titers ± standard deviations in groups of five mice.

‖ Significantly less than all other liver counts ($P < 0.001$).

¶ Significantly less than all other spleen counts ($P < 0.05$).

The dose of interferon administered was without effect on virus titers in the organs of recipients (Table III). On the other hand, hyperimmune serum achieved significant control of virus in both liver and spleen. However, this effect of immune serum seemed markedly less than the effects of immune spleen cells (Tables I, II) despite the fact that immune serum recipients possessed neutralizing antibody in their serum at a titer probably 10 times greater than immune spleen cell recipients.

A similar experiment showed that serum from donors immunized with avirulent virus 9 days before bleeding, conferred significantly less antiviral activity than hyperimmune serum. Therefore, hyperimmune serum was used

in further evaluation of the importance of antibodies in recovery. Direct comparison of the effects of hyperimmune serum and immune spleen cells will be described in a later section.

Transfer of Hypersensitivity to Ectromelia Virus Antigens with Immune Cells and Immune Serum.—The foregoing evidence suggested that the major antiviral activity of immune spleen cells was not related to the production of antibody or interferon in recipients. Therefore an experiment was performed to determine whether cells capable of mediating delayed hypersensitivity reactions to viral antigens were present in 6-day immune spleen cell populations, and to compare the characteristics of hypersensitivity reactions conferred upon recipients by immune cells and hyperimmune serum.

TABLE IV

Transfer of Hypersensitivity to Ectromelia Virus Antigens with Immune Spleen Cells or Hyperimmune Serum

Treatment of recipients	Hypersensitivity*			
	3 hr	6 hr	9 hr	24 hr
Immune cells i.v.‡	0	0.1 ± 0.2	0.1 ± 0.2	1.7 ± 0.4§
Immune serum i.v.	1.5 ± 0.7§	0.2 ± 0.4	0.1 ± 0.2	0.1 ± 0.2
Immune cells f.p.‡	0.4 ± 0.5	0.6 ± 0.4‖	1.0 ± 0.6§	0.7 ± 0.6

* Means of specific increases in foot thickness (in 0.1 mm units) ± standard deviations in groups of five mice.

‡ Harvested from donors immunized i.v. with 5×10^5 PFU avirulent ectromelia virus 6 days previously. Doses are given in the text. f.p., into the footpad.

§ Significant specific reaction ($P < 0.02$).

‖ $P < 0.05$.

A dose of 1.2×10^8 viable immune spleen cells/mouse was injected intravenously into a group of five mice; controls received 1.1×10^8 normal spleen cells. Another group of five mice was given hyperimmune mouse anti-ectromelia serum intravenously (0.2 ml/mouse); their control group received 0.2 ml of normal mouse serum. All mice were then tested by footpad inoculation for hypersensitivity to ectromelia virus antigens within 30 min of the receipt of cells or serum. A further group of five mice was injected in the right hind foot with 0.04 ml of a mixture of equal parts of the immune cell suspension (6×10^6 cells/mouse) and the virus antigen suspension; controls received a mixture of normal spleen cells and antigens.

The control groups provided a measure of the nonspecific foot swelling caused by injection trauma. The specific swelling due to hypersensitivity was then obtained by subtraction of control figures from those of the corresponding test groups to give the results shown in Table IV. Immune spleen cells conferred significant delayed (24 hr) hypersensitivity reactions upon recipients but no immediate (3 hr) hypersensitivity. Intravenous injection of cells resulted in the largest swellings occurring at 24 hr, while local injection into the foot caused acceleration of the reaction so that maximum readings were ob-

tained at 9 hr. In contrast, immune serum transfer produced significant immediate hypersensitivity but no delayed reactions. Thus, it is clear that the immune cell population contained effective mediators of CMI, but the transferred cells produced insufficient antibody to cause immediate hypersensitivity reactions to ectromelia antigens.

Specificity of Adoptive Immunity Conferred by Immune Spleen Cells.—If the antiviral activity of immune spleen cells depends upon CMI, it should be specific, i.e., immune cell populations with demonstrable specific activity against other antigens should be inactive against ectromelia virus, and vice versa. Therefore, spleen cells from mice immunized with *Listeria monocytogenes* were used in an experiment which was designed to test the specificity of ectromelia-immune cells.

A group of mice was immunized intravenously with 1.1×10^4 viable *Listeria*. Survivors were used 6 days later as donors of *Listeria*-immune spleen cells. Another group of mice donated ectromelia-immune spleen cells 6 days after intravenous immunization with 5×10^5 PFU avirulent virus. Six groups of four recipients were employed. Three groups were infected intravenously with 2×10^5 PFU virulent ectromelia virus 24 hr before cell transfer; the other three groups were infected intravenously with 1.5×10^5 viable *Listeria* at the time of cell transfer. One ectromelia-infected and one *Listeria*-infected group received 1.3×10^8 viable ectromelia-immune spleen cells intravenously/mouse; a second group from each infected pool received 1.4×10^8 viable *Listeria*-immune cells intravenously/mouse; the remaining groups served as controls for each infectious agent and were given no cells. Spleens of all *Listeria*-infected mice were removed for viable bacterial counts 24 hr after cell transfer. Livers were not taken since the spleen provides a more sensitive indication of the anti-*Listeria* activity of immune spleen cells (2). Spleens and livers of all ectromelia-infected mice were also removed at this time for virus titration.

Listeria-immune cells exhibited potent anti-*Listeria* activity but their anti-ectromelia activity was negligible (Table V). In contrast, ectromelia-immune cells were active against ectromelia virus but inactive against *Listeria*, thus demonstrating their specificity.

The Effects of ATS and Anti-Light Chain Serum upon the Antiviral Activity of Immune Spleen Cells.—Specifically commited lymphocytes mediating antibacterial resistance are inactivated by treatment with heterologous ATS (7). Therefore, similar cells responsible for antiviral mechanisms should also be susceptible to ATS. Furthermore, recent work in vitro (8) and in vivo (9) suggests that on the surfaces of the specifically sensitized lymphocytes active in CMI there are receptors for antigen which can be blocked by antibody directed against immunoglobulin light chains. Thus, if the antiviral activity of immune spleen cells is due to CMI, it should be blocked by anti-light chain serum. These predictions were tested in the following experiment.

A suspension of ectromelia-immune spleen cells in Puck's A saline was divided into four portions; to the first three portions, either anti-light chain serum, ATS, or normal rabbit serum (NRS) was added to give a final serum dilution of 1:4. These three suspensions were then

placed in an ice bath together with a fourth untreated control. All cell suspensions were removed from the ice bath after 2 hr, washed twice in Puck's A saline, examined for viability, and injected intravenously into groups of four preinfected recipient mice for each suspension. Because of the limited supply of anti-light chain serum, only small numbers of cells could be treated. Therefore, the dose of viable cells per recipient (2×10^7) was lower than in previous experiments. A fifth group of control recipients received no cells. All five groups were then sacrificed for virus titrations on their livers and spleens 24 hr after cell transfer.

Table VI indicates that the antiviral activity of immune spleen cells was abrogated by treatment with ATS or anti-light chain serum. Thus, these

TABLE V

Specificity of Adoptive Immunity Conferred by Immune Spleen Cells: Effects on Listeria monocytogenes and Ectromelia Virus Infections in Recipient Target Organs 24 Hr after Cell Transfer*

Immune cell‡ specificity	*Listeria*-infected§ mice	Ectromelia-infected mice‖	
	Spleen counts	Liver PFU	Spleen PFU
Listeria-immune	3.7 ± 0.2¶	7.3 ± 0.4	7.3 ± 0.4
Ectromelia-immune	6.3 ± 0.2	4.8 ± 0.7**	5.9 ± 0.7‡‡
Nil	6.6 ± 0.1	7.7 ± 0.3	7.6 ± 0.2

* As indicated by mean log viable bacteria per organ ± standard deviations, and mean log virus PFU per organ ± standard deviations in groups of four mice.

‡ Details of donor immunization and cell doses are given in the text.

§ *Listeria*-infected recipients were given 1.5×10^5 viable *Listeria* i.v. at the time of cell transfer.

‖ Ectromelia-infected recipients were given 2×10^5 PFU virulent virus i.v. 24 hr before cell transfer.

¶ Significantly less than both other groups ($P < 0.001$).

** $P < 0.01$.

‡‡ $P < 0.05$.

results are consistent with the contention that the major antiviral effect of immune spleen cells depends upon specifically sensitized lymphocytes.

Effect of Anti-Theta Ascitic Fluid on the Antiviral Activity of Immune Spleen Cells.—Antibodies against the thymus-specific, theta antigen (10) afford a means of defining the roles of thymus-derived lymphocytes in immune phenomena. Recent reports suggest that the specifically sensitized effector cells in CMI are thymus-derived (11, 12). Therefore, if antiviral activity conferred by immune spleen cells is CMI-dependent, it should be absent from cell populations in which the thymus-derived cells have been inactivated. Anti-theta ascitic fluid was used to test this possibility.

Equal numbers of spleen cells from 6-day immune CBA donors were suspended in either anti-theta ascitic fluid or normal mouse serum (NMS) and incubated at 37°C for 30 min. The cells were then washed twice in Puck's A saline, resuspended in a 1:2 dilution of guinea pig

complement in Hanks' balanced salt solution, and again incubated at 37°C for 30 min. These two suspensions, together with a third control suspension which had been kept in an ice bath, were washed twice in Puck's A saline, examined for viability, and injected intravenously into groups of four preinfected CBA recipients. Apparent viability of complement-treated cells was

TABLE VI

Effects of Anti-Light Chain Serum and ATS on the Antiviral Activity of Immune Spleen Cells*

Treatment of immune cells‡	Posttreatment % viability of intact cells	Dose of viable cells/mouse	Virus titers 24 hr after cell transfer	
			Liver	Spleen
Anti-light chain serum	62	1.9×10^7	7.9 ± 0.3§	7.0 ± 0.2‖
ATS	73	2.1×10^7	8.1 ± 0.2¶	7.0 ± 0.1**
NRS	72	2.0×10^7	7.2 ± 0.4	6.2 ± 0.5
Nil	70	1.9×10^7	7.0 ± 0.3	5.7 ± 0.4
—	—	Nil	7.9 ± 0.4	7.2 ± 0.4

* As indicated by mean log PFU per organ ± standard deviations in groups of four recipients infected i.v. with 2×10^5 PFU virulent ectromelia virus 24 hr before cell transfer.

‡ Harvested from donors immunized i.v. with 5×10^5 PFU avirulent ectromelia virus 6 days previously. Treatment details are given in the text.

§ Significantly greater than "Nil" ($P < 0.02$).

‖ Significantly greater than "NRS" ($P < 0.05$) and "Nil" ($P < 0.02$).

¶ Significantly greater than "NRS" ($P < 0.02$) and "Nil" ($P < 0.01$).

** Significantly greater than both "NRS" and "Nil" ($P < 0.02$).

TABLE VII

Effect of Anti-Theta Ascitic Fluid on the Antiviral Activity of Immune Spleen Cells*

Treatment of immune cells‡	Posttreatment % viability of intact cells	Dose of viable cells/mouse	Virus titers 24 hr after cell transfer	
			Liver	Spleen
Anti-theta ascitic fluid	96	1.3×10^7	8.3 ± 0.1§	9.4 ± 0.4§
NMS	96	1.1×10^7	6.6 ± 0.7	8.2 ± 0.6
Nil	82	1.6×10^7	6.8 ± 0.7	8.4 ± 0.3
—	—	—	8.2 ± 0.5	9.5 ± 0.2

* As indicated by mean log PFU per liver or spleen ± standard deviations in groups of four recipients infected i.v. with 2×10^5 PFU virulent ectromelia virus 24 hr before cell transfer.

‡ Harvested from donors immunized i.v. with 5×10^5 PFU avirulent ectromelia virus 6 days previously. Treatment details are given in the text.

§ Significantly greater than "NMS" ($P < 0.02$) and "Nil" ($P < 0.05$).

abnormally high, probably because dead cells were completely lysed and thus not detectable by dye uptake. As in the previous experiment, only a small number of cells could be treated in the volume of anti-theta ascitic fluid available, so that the dose of viable cells per recipient was $1–2 \times 10^7$. A fourth group of control CBA recipients were given no cells. Virus titrations were performed on the livers and spleens of all recipients 24 hr after cell transfer.

Immune cell populations treated with anti-theta antibodies completely lost their antiviral activity (Table VII), a result consistent with an essential role for thymus-derived lymphocytes.

Effect of Irradiation on Antiviral Mechanisms in Recipients of Immune Spleen Cells or Hyperimmune Serum.—Blood monocytes appear to be essential for the development of CMI-dependent antibacterial resistance in foci of infection caused by intracellular bacterial parasites (13, 14). Therefore, it seemed reasonable to expect that antiviral mechanisms would also involve monocytes. Irradiation doses as low as 400 rads cause severe depletion of blood monocytes within 24 hr (15). This effect was utilized in the following experiment.

A group of 20 mice was given 800 rads whole body gamma-irradiation immediately before intravenous infection with 2×10^5 PFU virulent ectromelia virus. After 24 hr, subgroups of five mice together with groups of five unirradiated, infected controls were injected intravenously with 1.2×10^8 viable ectromelia-immune spleen cells/mouse, or with hyperimmune anti-ectromelia serum (0.2 ml/mouse). After a further 24 hr these mice were sacrificed for virus titration of their livers and spleens, and serum neutralizing antibody determinations. The 10 remaining irradiated, infected mice and 10 unirradiated, infected mice served as controls receiving neither immune cells nor immune serum; five mice of each group were sacrificed for titration of virus in the liver and spleen at the time of cell and serum transfer, and the remaining five were used 24 hr later.

Irradiated mice given no other treatment had significantly higher virus titers in the liver ($P < 0.01$) than unirradiated controls both at the time of cell or serum transfer and 24 hr later, i.e. 24 and 48 hr after infection, whereas spleen titers were not significantly different ($P > 0.10$) (Table VIII). Irradiation of recipients almost completely repressed the expression of antiviral activity conferred by immune serum. This effect was less complete in recipients of immune cells. The significance of repression was determined by an analysis of variance test comparing the changes in virus titers in irradiated and unirradiated recipients over the 24 hr period after adoptive immunization. Repression of the effects of both immune cells and immune serum was highly significant in the spleen ($P < 0.01$) and liver ($P < 0.001$). These results are readily explained by the assumption of an important role for recipient monocytes in the expression of antiviral activity. The less complete repression in the recipients of immune cells could then be due to either the direct antiviral activity of specifically sensitive lymphocytes, or to partial reconstitution of recipient monocyte levels. The latter possibility seems unlikely since there is evidence that the spleen is not a significant source of monocytes in other systems (14, 16–18).

Finally, immune spleen cells were far more efficient in controlling virus multiplication in recipient target organs than high titer immune serum, although neutralizing antibody was undetectable in the sera of cell recipients and was present at high levels in sera of immune serum recipients.

The Antiviral Activity of an Immune Spleen Cell Population Depleted of Mononuclear Phagocytes.—The data presented to this point imply that thymus-derived, specifically sensitized lymphocytes play an essential role in the passive transfer of antiviral activity by immune spleen cells, but indicate that full expression of this activity may depend upon recipient monocytes. The following experiment was performed to determine whether significant antiviral activity also resided in mononuclear phagocytes present in immune cell populations.

TABLE VIII

Effect of Irradiation on Antiviral Mechanisms in Recipients of Immune Spleen Cells or Hyperimmune Serum*

Treatment of recipients‡	Time after cell or serum injections						
	0 hr		24 hr				Neutralizing antibody§
	Liver	Spleen	Liver		Spleen		
			PFU	P‖	PFU	P‖	
Irradiation + immune cells	—	—	4.8 ± 1.3	<0.01	5.6 ± 0.6	<0.05	<10
Irradiation + immune serum	—	—	7.3 ± 0.2	<0.01	6.9 ± 0.2	N.S.¶	224 ± 88
Irradiation	5.6 ± 0.2	4.9 ± 0.6	7.9 ± 0.1	—	6.9 ± 0.2	—	—
Immune cells	—	—	1.5 ± 0.3	<0.001	2.9 ± 1.0	<0.01	<10
Immune serum	—	—	4.6 ± 1.0	<0.01	4.4 ± 1.0	<0.01	256 ± 88
Nil	4.9 ± 0.2	4.4 ± 0.5	6.9 ± 0.4	—	6.7 ± 0.4	—	—

* As indicated by mean log PFU per liver or spleen ± standard deviations in groups of five recipients infected i.v. with 2×10^5 PFU virulent ectromelia virus 24 hr before cell and serum transfer.

‡ Immune spleen cells were harvested from donors immunized i.v. with 5×10^5 PFU avirulent ectromelia virus 6 days previously. Doses of immune cells and immune serum, and irradiation details are given in the text.

§ Reciprocals of titers ± standard deviations in groups of five mice.

‖ Values of probability, P, derived from t test of the significance of the difference between means of test groups and appropriate irradiated or untreated control group.

¶ N.S., not significant ($P > 0.05$).

A crude suspension of 6-day immune spleen cells was dispersed by pipetting, passed once through loosely packed cotton to remove remaining clumps, washed once in Puck's A saline, and divided into two portions. One suspension was passed seven times through tightly packed 3×0.5 cm cotton columns at 20°C while the other remained in an ice bath. When samples of the two suspensions were cultured in Eagle's medium with 10% fetal calf serum, it was found that the seven passages through cotton columns had eliminated mononuclear phagocytes, i.e., mononuclear cells capable of adherence and spreading on glass. The two cell suspensions were each injected intravenously into groups of four preinfected recipients. Virus titrations on recipient livers and spleens were performed 24 hr later.

The results in Table IX indicate that rigorous removal of mononuclear phagocytes from the immune spleen cell population did not impair its antiviral activity.

DISCUSSION

Recovery from mousepox depends upon the control of virus growth in the liver (5). Therefore, passive transfer experiments were designed to investigate the antiviral mechanisms involved in this process.

Preliminary experiments showed that the spleens of donor mice which had been immunized intravenously with avirulent virus contained cells with significant antiviral potential at least as soon as the 4th day. Immune cell activity was maintained virtually unchanged until the 10th day but waned significantly by day 20. These results are in agreement with previous evidence (1) which indicated that recovery mechanisms began to operate in the spleen and liver 4–6 days after subcutaneous infection with virulent virus.

TABLE IX

The Antiviral Activity of an Immune Spleen Cell Population Depleted of Mononuclear Phagocytes*

Treatment of immune cells‡	Dose of viable cells/mouse	Virus titers 24 hr after cell transfer	
		Liver	Spleen
Monocyte depletion	5.3 ± 10^7	3.0 ± 0.2§	2.4 ± 0.4‖
Nil	5.1×10^7	3.0 ± 0.3§	2.5 ± 0.5‖
—	—	7.2 ± 0.3	7.2 ± 0.9

* As indicated by mean log PFU per liver or spleen ± standard deviations in groups of four recipients infected i.v. with 2×10^5 PFU virulent ectromelia virus 24 hr before cell transfer.

‡ Harvested from donors immunized i.v. with 5×10^5 PFU avirulent ectromelia virus 6 days previously. The monocyte depletion procedure is described in the text.

§ Less than control ($P < 0.001$).

‖ $P < 0.01$.

Immune cells did not contain preformed factors which could account for their activity, since sonically disrupted cells were inactive (Table I). In addition, antiviral activity was not accompanied by the production of detectable neutralizing antibody or splenic interferon in immune cell recipients (Table II). Passively administered interferon was ineffective (Table III), while immune sera conferred less antiviral activity than immune cells (Tables I–III and VIII) but higher neutralizing antibody titers (Tables II, III, and VIII). In fact, the neutralizing antibody titers of hyperimmune serum recipients were far greater than those generated in mice which had recovered from primary infection (1). However, this level of serum antibody only prevented further multiplication of virus over a 24 hr interval, and did not cause a significant drop in titers (Tables III, VIII).

The foregoing data contraindicate important roles for interferon and neutralizing antibody in recovery from mousepox, but support evidence presented

in an earlier paper (1) which suggested an essential role for CMI in the recovery process. Further evidence on this question was obtained in the present experiments and is summarized in the following five points.

(*a*) The cellular mediators of delayed hypersensitivity to virus antigens were present in immune spleen cell populations, since mice given these cells intravenously mounted significant delayed reactions when tested in the foot (Table IV). Cells secreting free antibody to virus antigens could have been present in this immune population, but their activity was not sufficient to produce immediate hypersensitivity reactions in recipients, even when the cells were injected directly into the test site together with antigens. In contrast, mice which received an intravenous injection of hyperimmune serum gave significant immediate reactions.

(*b*) The antiviral mechanism conferred by immune cells was specific (Table V).

(*c*) Complete inhibition of the antiviral activity of immune cells was achieved by treatment in vitro with ATS, or anti-theta ascitic fluid plus complement (Tables VI, VII), thus suggesting that the effective cells in the population were thymus-derived. This conforms with the results of Williams and Waksman (11) and Cerottini, Nordin, and Brunner (12) who have recently obtained results which suggest that the specifically sensitized cells in CMI in other systems are thymus-derived. Furthermore, this evidence argues against an important antiviral function for antibody-secreting cells. These cells are not thymus-derived lymphocytes in systems so far elucidated in the mouse (19, 20).

(*d*) Treatment of immune cells with rabbit anti-mouse light chain serum completely inhibited their antiviral activity (Table VI). There is evidence that the specificity of sensitized lymphocytes depends upon an immunoglobulin-like surface receptor for antigen and that heterologous antisera directed against the light chains of immunoglobulins may block these receptors, thus rendering the cells unresponsive to antigen (8, 9).

(*e*) Extensive filtration of immune spleen cell populations which removed all mononuclear phagocytes left antiviral activity unimpaired (Table IX).

In summary, these results strongly suggest that specifically sensitized, thymus-derived lymphocytes are mainly responsible for the efficient mechanisms of recovery transferred by immune spleen cells. However, they do not elucidate the nature of these antiviral mechanisms. The use of recipients which had been irradiated (800 rads) 24 hr before adoptive immunization provided information on this question. These animals were unable to express fully the antiviral activity of immune spleen cells or hyperimmune serum (Table VIII), thus indicating that radio-sensitive recipient components played an important role.

The available evidence suggests that blood monocytes are the recipient components concerned. These cells are generated mainly in bone marrow by the

division of radiosensitive progenitors (16, 17). Within 24 hr of irradiation there is marked depletion of blood monocytes and almost complete suppression of delayed-hypersensitivity reactions (15) in which bone marrow-derived cells comprise the bulk of the cellular infiltration (18, 21). Irradiation also suppresses the development of CMI-dependent antibacterial resistance which requires a monocytic invasion of infectious foci (13, 14). Therefore, it seems likely that in the normal recipient mice used in the present experiments, a monocytic invasion of virus-infected foci, triggered by specifically sensitized lymphocytes, occurred over the 24 hr interval after immune cell transfer. After immune serum transfer, monocytes might enter infectious foci as a sequel to acute inflammation (17, 22) provoked by antigen–antibody complexes, or possibly as a result of passive sensitization by adsorbed cytophilic antibody (23).

The possible roles of lymphocytes and monocytes in the recovery process will be discussed further in the accompanying paper (24).

SUMMARY

The following passive transfer experiments evaluated the contributions of the various host responses in recovery from mousepox. (*a*) Immune spleen cells transferred highly efficient antiviral activity, but preinfected recipients of these cells made no detectable splenic interferon or antibody in the 24 hr interval after cell transfer. (*b*) Passively administered interferon was ineffective. (*c*) Recipients of hyperimmune serum had much more antibody than recipients of immune spleen cells but significantly less antiviral activity. (*d*) Immune spleen cell populations with antiviral activity contained mediators of CMI to virus antigens. (*e*) The antiviral activity of immune spleen cells was specific; it was inhibited by in vitro treatment with ATS, anti-light chain serum, and anti-theta ascitic fluid, but not by removal of mononuclear phagocytes from the immune population.

These results are interpreted to mean that recovery mechanisms conferred by immune spleen cells were triggered by specifically sensitized, thymus-derived lymphocytes, and that antibody and interferon responses were of less importance. A radiosensitive recipient component was necessary for the full expression of the antiviral activity of both immune cells and immune serum. It seemed likely that this component was the blood monocyte.

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