MECHANISMS OF RECOVERY FROM A GENERALIZED VIRAL INFECTION: MOUSEPOX

III.   REGRESSION OF INFECTIOUS FOCI

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The preceding paper in this series (1) showed that immune spleen cell populations containing lymphocytes active in cell-mediated immunity (CMI)[1] conferred dramatic antiviral activity upon the livers and spleens of preinfected recipients, while immune serum was much less effective. However, radiosensitive recipient components were necessary for the full expression of the antiviral mechanisms triggered by both CMI and antibodies. It seemed likely that these components were blood monocytes[2] which were attracted into infectious foci.

This report presents evidence that a mononuclear cell invasion of infectious foci in the liver accompanies the regression of infection in that organ and evaluates potential antiviral mechanisms in the focus.

Materials and Methods

General.—Details of the stocks of ectromelia virus and hyperimmune anti-ectromelia serum, and assays for virus plaque-forming units (PFU), neutralizing antibody, interferon, and hypersensitivity to viral antigens were given in the first paper in this series (2). The second paper (1) described the strain of *Listeria monocytogenes*, methods for its enumeration in infected tissues, and the preparation of mouse spleen cell suspensions.

Animals.—Inbred C57BL, C3H, and CBA mice, and C57BL $\times$ C3H F_1 hybrids were obtained from breeding colonies in this laboratory. Mice of the same age, sex, and strain were used in the test groups of individual experiments.

Immunofluorescence.—Liver pieces were frozen in glass tubes immersed in liquid N_2. 6 μ sections were cut on a cryostat, thoroughly dried, and fixed in acetone for 10 min. After further drying, sections were stained for 20 min with fluorescein-conjugated rabbit immunoglobulins specific for ectromelia virus antigens, using 10% rhodamine bovine albumin as a counterstain. The specimens were then washed for 20 min with phosphate-buffered saline, and mounted in neutral glycerol for examination and photography.

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[1] *Abbreviations used in this paper:* CMI, cell-mediated immunity; GVHR, graft-*versus*-host reactions; PFU, virus plaque-forming units.

[2] "Monocytes" will be used to describe mononuclear phagocytes in the blood, and "macrophages" will describe mononuclear phagocytes in tissues or in the peritoneal cavity. The term "mononuclear cells" will encompass both phagocytic cells and lymphocyte-like cells.

Effects of Immune Spleen Cells and Immune Serum on the Histopathology of the Livers of Infected Recipients.—

The animals used in the histological and immunofluorescence studies described here were C57BL recipient mice from some of the quantitative passive transfer experiments reported in the preceding paper (1). All of these animals were infected intravenously with 2×10^5 PFU virulent ectromelia virus. 24 hr later, when progressive infection was established in hepatic parenchyma, groups of mice were injected intravenously with one of the following: 10^8 viable spleen cells from donors which had been immunized intravenously with 5×10^5 PFU avirulent virus 6 days previously, 10^8 viable normal spleen cells, 0.2 ml of hyperimmune anti-ectromelia mouse serum, or 0.2 ml of normal mouse serum. An additional control group was left untreated. Livers of four or five mice from each group were removed at intervals of 10 and 24 hr after the injection of cells or serum. One small piece of each liver was fixed in 10% formalin in saline, processed for histology, and stained with hematoxylin and eosin; a second piece was frozen in liquid N_2 for immunofluorescence examination.

Histologically, small necrotic lesions were present in the hepatic parenchyma of all mice which received normal cells, normal serum, or were left untreated. These pale-staining, eosinophilic areas were presumably the sites of virus infection. No focal mononuclear cell infiltration was present, either in the necrotic lesions or elsewhere.

In contrast, mononuclear cell foci were clearly visible in the livers of all the mice which had received immune cells or immune serum. 10 hr after adoptive immunization, mononuclear cells appeared to be invading and destroying the foci of necrotic parenchymal cells; 14 hr later, no necrotic areas without mononuclear cell infiltration were seen (Fig. 1). Lesions resulting from immune cell transfer were more densely populated with mononuclear cells than those caused by hyperimmune serum (Fig. 2). Many of the cells appeared to be macrophages.

Immunofluorescence examination confirmed that the mononuclear cells had invaded virus-infected lesions. Scattered foci of infected hepatic parenchyma cells were visible in the livers of all mice which received normal cells, normal serum, or were left untreated. However, in adoptively immunized mice, invasion of infected foci by mononuclear cells (identifiable by phase contrast) occurred, and the amount of fluorescent material in all of the lesions was markedly reduced. The apparent destruction of infected hepatic cells was more nearly complete in immune cell recipients than in hyperimmune serum recipients (Fig. 3). Some of the remaining fluorescence in both cases resided in cells which were possibly eosinophilic granulocytes; these cells stained nonspecifically with control conjugates. No fluorescence was observed in mononuclear cells. Thus, the destruction of virus-infected cells and virus antigens could well have proceeded to completion in some lesions.

These results, together with the previous demonstration of the specificity of passively administered immune cells (1), suggested that mononuclear cell
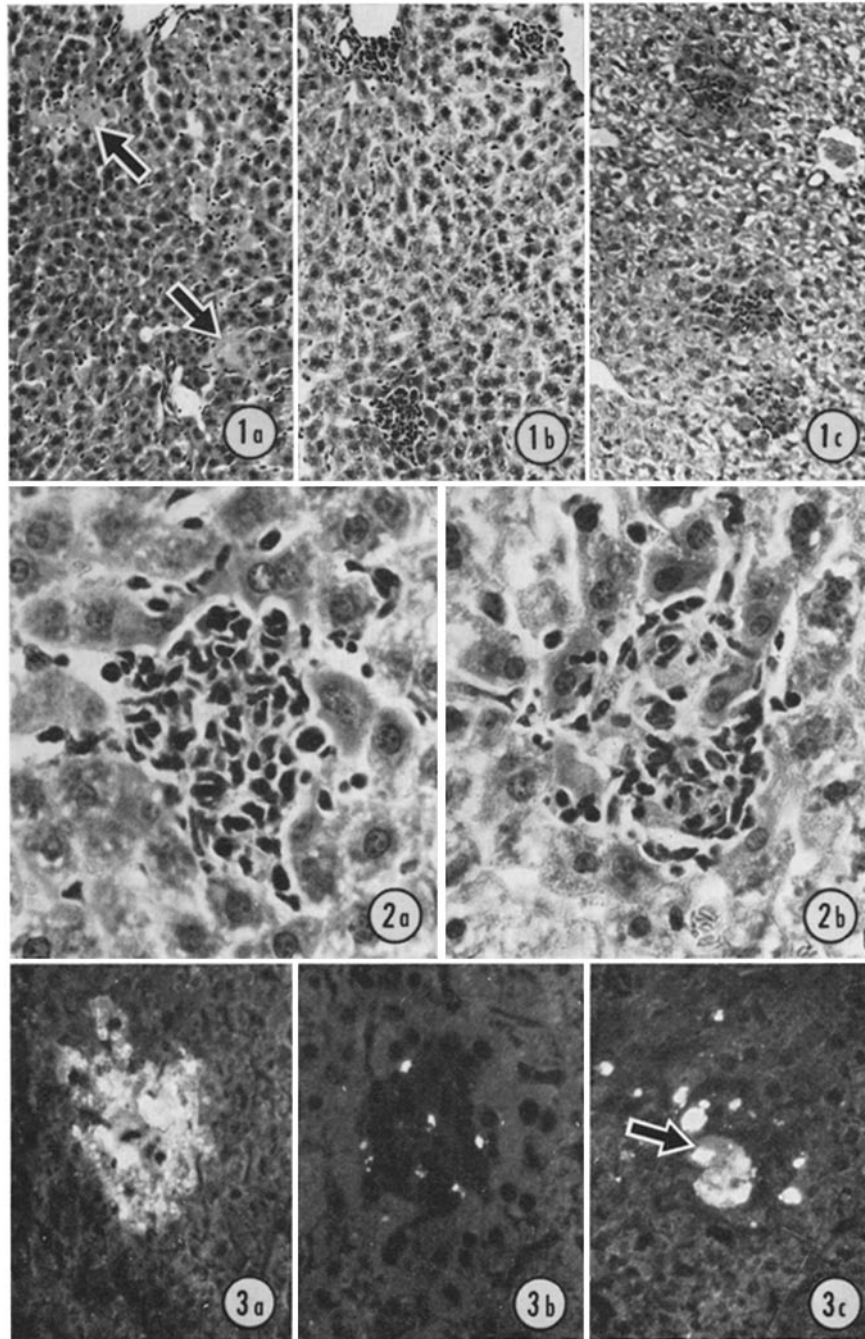
entry into infectious foci was triggered by immune mechanisms. An important question now arises. Does a similar mononuclear cell invasion of infected areas in the liver occur under immune direction during natural recovery from a primary infection in normal mice?

The earlier papers in this series (1, 2) indicated that mechanisms essential for recovery from primary mousepox depended upon CMI, but the present studies show that antibodies, while less efficient than CMI, can also trigger a mononuclear cell invasion of infectious foci which accompanies regression of infection. Therefore, an unequivocal answer to the question posed above would require experimental animals with immunodepression affecting both cell-mediated and humoral responses but intact monocyte-macrophage activity; liver histology in these mice could then be compared with that in normal controls during recovery from primary mousepox. Mice with graft-*versus*-host reactions (GVHR) were previously shown to have properties which make them most suitable for such experiments (3), for if tested at a certain phase of the reaction, they have severe immunosuppression with concurrent nonspecific hyperactivity of their macrophages.

Relevant Features of GVHR.—Preliminary investigations showed that doses of 7×10^7–2×10^8 viable spleen cells from C57BL donors given intravenously to C57BL $\times$ C3H F_1 hybrid recipients produced suitable GVHR. The C57BL cell injection caused a splenomegaly (characteristic of GVHR) which reached a peak after 10 days and then slowly subsided. There was no apparent debility or change in body weight. Histology of the spleen and liver was variable, but did not confuse the interpretation of histopathologic changes caused by mousepox.

Effect of GVHR on Resistance to Infection with Listeria monocytogenes.— Infection with *Listeria monocytogenes*, an intracellular bacterial parasite, is a sensitive indicator of macrophage activity in the mouse (3). Therefore, 13 days after initiation of GVHR in F_1 hybrid mice by the intravenous injection of

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Fig. 1. Sections of livers taken 48 hr after i.v. injection with 2×10^5 PFU virulent ectromelia virus and 24 hr after the receipt of normal spleen cells (*a*), immune spleen cells (*b*), or hyperimmune serum (*c*). Two necrotic lesions are indicated by arrows in the section from a recipient of normal cells. Three mononuclear cell foci are visible in the sections from recipients of immune cells and immune serum. $\times$ 125.

Fig. 2. Typical liver lesions 48 hr after virus infection and 24 hr after receipt of immune spleen cells (*a*), or hyperimmune serum (*b*). Mononuclear cells are more densely packed in the lesion resulting from immune cell transfer. $\times$ 500.

Fig. 3. Liver lesions 48 hr after virus infection and 24 hr after receipt of normal spleen cells (*a*), immune spleen cells (*b*), or hyperimmune serum (*c*), in preparations stained with fluorescein-conjugated rabbit anti-ectromelia globulin. The fluorescent (infected) hepatic cells seen in the normal cell recipient have been eliminated from the recipient of immune cells. Some fluorescent hepatic cells, indicated by the arrow, have escaped destruction in the hyperimmune serum recipient. The small, brightly fluorescent cells are probably eosinophilic granulocytes. $\times$ 320.

7.6×10^7 viable C57BL spleen cells/mouse, 12 mice with GVHR and 12 normal control mice were infected intravenously with 2.5×10^4 viable *Listeria*. At this time, mean spleen weight in a group of four mice with GVHR was 143 mg, compared with 57 mg in a group of four controls.

One of the mice with GVHR died from the *Listeria* infection whereas eight controls succumbed. This protective effect of GVHR was significant ($\chi^2 = 15.2$, $P < 0.001$), and thus conforms with previous results (3) which showed that mice with GVHR were more resistant to *Listeria* infection because their macrophages were more efficient in killing ingested organisms. Histologically, large numbers of macrophages were present in infectious foci in the livers of all mice (both with and without GVHR) within 24 hr of infection, thus indicating that immune mechanisms were not essential for their entry into lesions. Since the experiments of North (4) suggest that macrophages which populate liver lesions in *Listeria* infection are derived from blood monocytes, the present results indicated that monocyte–macrophage activity in mice with GVHR was superior to that in normal mice.

Effects of GVHR on the Delayed-Hypersensitivity and Antibody Responses to Ectromelia Virus.—

GVHR were initiated in a group of F_1 hybrid mice by the intravenous injection of 1.0×10^8 viable C57BL spleen cells/mouse. Controls were left untreated. 10 days later, the spleens of four mice of each group were removed and weighed. Mean spleen weight was 225 mg in mice with GVHR and 68 mg in controls. 10 mice of each group were then infected subcutaneously over the thorax with 5×10^5 PFU avirulent virus. This dose of avirulent virus was chosen so that the mice would survive long enough for their immune responses to be measured (1). On days 6 and 9 after infection, separate subgroups of five mice with GVHR and five control mice were tested for immediate and delayed hypersensitivity to virus antigens by footpad inoculation. Additional subgroups of five mice which had not been infected were included in this test as controls indicating nonspecific reactivity on each day. All infected mice in the test groups were bled for neutralizing antibody assays after the 24 hr hypersensitivity readings, i.e., on days 7 and 10 after infection. Their livers and spleens were also removed for virus titration at this time.

The GVHR caused an immunosuppression involving both cell-mediated (24 hr hypersensitivity) and humoral (3 hr hypersensitivity and neutralizing antibody) responses (Table I). This was the probable cause of the increase of virus titers in the liver and spleen from the 7th to 10th day in mice with GVHR. These results suggested that macrophage hyperactivity which protected mice with GVHR from *Listeria* infection was not similarly effective against mousepox, presumably because of the suppression of immune mechanisms needed for the attraction of mononuclear cells to virus-infected foci. This question was then examined in detail in the following experiment in which virulent virus was used in order to simulate the natural infection (5).

The Effects of GVHR on Primary Mousepox.—

A group of F_1 hybrid mice were injected intravenously with 1.2×10^8 viable C57BL spleen cells/mouse. Controls were not treated. After 12 days, spleens of four mice of each group were

weighed; mean spleen weight was 229 mg in mice with GVHR and 83 mg in controls. 26 mice of each group were then infected subcutaneously in the right hind foot with 10^8 PFU virulent ectromelia virus. 10 mice of each group were set aside to assess mortality over a 14 day interval, and 4 mice of each group were sacrificed 4, 6, 7, and 8 days after infection. PFU in liver, spleen, and blood cells, and serum neutralizing antibody titers were determined for individual mice. Small pieces of the livers of all mice were fixed for histological examination, and the spleen homogenates remaining after virus titration on each of days 4, 6, and 7 were used for interferon assays.

Mortality in mice with GVHR was 80%, while in controls it was only 20%. This difference was significant ($P < 0.05$). The trend of infection in liver, spleen, and blood (Table II) was in accord with the mortality data. Mice with GVHR had progressively increasing virus titers from the 4th to 8th days;

TABLE I

Effects of GVHR on the Delayed-Hypersensitivity and Antibody Responses after Subcutaneous Infection with 5×10^5 PFU Avirulent Ectromelia Virus

Day after nfection	Treatment	Hypersensitivity*			Neutra-lizing antibody‡	PFU§	
		3 hr	6 hr	24 hr		Liver	Spleen
6	GVHR‖	0	0.2 ± 0.3	0.1 ± 0.2	—	—	—
	Control	0	0.5 ± 0.4	2.5 ± 0.7¶	—	—	—
7	GVHR	—	—	—	<5	2.6 ± 0.9	4.3 ± 0.7
	Control	—	—	—	<5	<2.4	1.6 ± 1.1
9	GVHR	0.7 ± 0.8	0.9 ± 0.5	0.3 ± 0.3	—	—	—
	Control	1.6 ± 1.0	2.4 ± 1.3	4.1 ± 2.3**	—	—	—
10	GVHR	—	—	—	<5	4.5 ± 0.7	6.1 ± 0.6
	Control	—	—	—	14 ± 5.5	<2.4	<0.7

* Means of increases in foot thickness (in 0.1 mm units) ± standard deviations in groups of five mice.
‡ Means of reciprocals of titers ± standard deviations in groups of five mice.
§ Mean log PFU per organ ± standard deviations in groups of five mice.
‖ GVHR initiated by i.v. injection of 10^8 parental strain spleen cells 10 days before infection.
¶ Significantly more than GVHR group ($P < 0.01$).
** $P < 0.05$.

these became significantly higher than control titers on the 7th and 8th days when means were compared in t tests (deaths began to occur on day 8). On the other hand, in control mice, virus titers on the 6th day were no higher than on day 4 and tended to be lower on days 7 and 8. One mouse with high titers, presumably an animal destined to die, caused the 8-day means to be higher than those on day 7.

In striking contrast to the later days, the 4th day virus titers in GVHR mice were significantly lower than in controls. This suggested that hyper-activity of macrophages caused by the GVHR may have resulted in improved protection of the liver and spleen from initiation of infection by blood-borne virus during the primary viremia (5).

The splenic interferon response seemed unimpaired by GVHR and appeared to be directly related to virus titers in the spleen as indicated by earlier work

(1, 2). However, neutralizing antibody titers were markedly depressed in mice with GVHR.

Histological examination showed that necrotic lesions in the livers of mice

TABLE II

The Effects of GVHR on the Course of Infection* after Subcutaneous Inoculation of 10²
PFU Virulent Ectromelia Virus

Day after infection	Treatment	Liver	Spleen	Blood cells	Interferon‡ (units per spleen)	Neutralizing antibody§
4	GVHR‖	2.9 ± 1.0¶	2.3 ± 1.1¶	<1.5	0	—
	Control	5.3 ± 0.4	5.6 ± 0.6	1.5 ± 0.5	13	—
6	GVHR	5.4 ± 0.4	5.2 ± 1.0	1.8 ± 0.8	3	<5
	Control	5.6 ± 1.3	5.3 ± 1.8	1.9 ± 0.5	0	<5
7	GVHR	6.0 ± 0.3¶	5.5 ± 0.4¶	2.5 ± 0.3	10	<5
	Control	4.2 ± 0.5	3.3 ± 1.0	2.0 ± 0.2	0	12 ± 5
8	GVHR	8.3 ± 0.9¶	6.7 ± 0.5¶	4.5 ± 1.9¶	—	<5
	Control	4.6 ± 1.6	5.1 ± 1.3	2.9 ± 1.4	—	65 ± 30

* As indicated by mean log PFU in liver, spleen, and blood cells ± standard deviations in groups of four mice.
‡ Derived from pools of four spleens.
§ Means of reciprocals of titers ± standard deviations in groups of four mice.
‖ GVHR initiated by i.v. injection of 1.2 × 10⁸ parental strain spleen cells 12 days before infection.
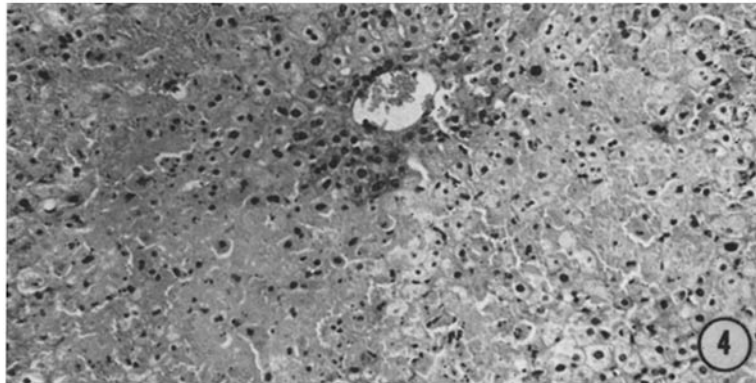¶ Significantly different from control ($P < 0.05$).

FIG. 4. Liver section of a mouse with graft-*versus*-host reaction taken 8 days after subcutaneous infection with 10² PFU virulent ectromelia virus. Many of the liver parenchyma cells are necrotic; in other areas the cells are grossly swollen. One small patch of normal-looking liver tissue can be seen adjacent to a central vein. × 125.

with GVHR became larger from the 6th to 8th days after infection; there was no apparent invasion of these areas by mononuclear cells. In some 8-day sections, lesions were almost confluent, and remaining parenchyma cells were grossly swollen (Fig. 4). Such lesions were also present in the control mouse with a high virus titer in the liver on day 8.

In contrast, by the 6th day the necrotic lesions of most control mice were being invaded by mononuclear cells which ranged in morphology from small lymphocytes to macrophages (Fig. 5). Further invasion of the foci appeared to be taking place on the 7th and 8th days. Over this time interval, most of the lesions became smaller and more densely populated with mononuclear cells as the necrotic debris disappeared.

Thus, in combination with the data presented in Tables I and II, this histological evidence indicated that the control of infection in the liver, which is essential for recovery from primary mousepox, was accompanied by an invasion of infectious foci by mononuclear cells under immune direction.

The means by which macrophages and lymphocytes may cause regression of infection now becomes a key question. Further elucidation of the activities
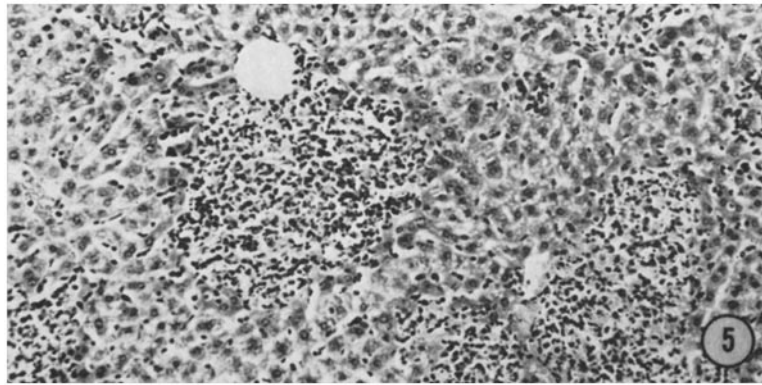

Fig. 5. Liver section of a normal mouse taken 6 days after subcutaneous infection with 10^2 PFU virulent ectromelia virus. Mononuclear cell foci of various sizes are visible. $\times$ 125.

of lymphocytes awaits the development of more carefully defined experimental systems. The possible antiviral activities of macrophages within infectious foci are also difficult to evaluate. Furthermore, virus yields from ectromelia-infected macrophages are so small that quantitative studies cannot be adequately made in vitro (6). Therefore, in the present investigation, further information on this question was obtained from experiments on normal or nonspecifically stimulated macrophage populations in the liver and spleen.

Antiviral Activity of Hyperactive Macrophages.—The preceding experiment suggested that hyperactive littoral macrophages in the livers and spleens of mice with GVHR may be more refractory to ectromelia virus than normal. Therefore, this possibility was tested by intravenous injection of virus which results in its rapid uptake by littoral macrophages in the liver and spleen (7).

GVHR were initiated in a group of F_1 hybrid mice by intravenous injection of 1.3 $\times$ 10^8 viable C57BL spleen cells. 9 and 11 days later, spleens from subgroups of four mice were frozen

and processed for interferon assays. These spleens were approximately three times larger than those of normal control mice. Also on day 11, 20 remaining mice with GVHR and 20 untreated controls were injected intravenously with 2×10^5 PFU virulent ectromelia virus. Four mice from each group were bled from the retro-orbital plexus 5 min after intravenous injection, and their livers and spleens were removed and frozen in liquid N_2 within 7 min of injection. Livers and spleens of further groups of four mice were removed after 4, 10, 24, and 48 hr. Virus titers in individual bloods and organs were determined.

Over 95% of the injected dose of virus was cleared from the blood within 5 min (Table III). Clearance was significantly enhanced in mice with GVHR. Only about 10% of virus was recovered from the liver after 7 min; the spleen

TABLE III

The Effects of GVHR on the Distribution and Behavior of Virulent Ectromelia Virus* after I.V. Injection of 2×10^5 PFU

Time after infection	Treatment	Blood	Liver	Spleen
5 min	GVHR‡	2.7 ± 0.6§	—	—
	Control	3.8 ± 0.4	—	—
7 min	GVHR	—	4.2 ± 0.2	2.4 ± 0.2§
	Control	—	4.4 ± 0.1	2.8 ± 0.1
4 hr	GVHR	—	<2.1	<0.7
	Control	—	<2.1	<0.7
10 hr	GVHR	—	2.4 ± 0.3§	2.2 ± 0.5
	Control	—	3.3 ± 0.3	3.2 ± 0.4
24 hr	GVHR	—	5.1 ± 0.2	4.7 ± 0.4§
	Control	—	5.6 ± 0.4	5.5 ± 0.2
48 hr	GVHR	—	6.7 ± 0.3	7.6 ± 0.2
	Control	—	7.0 ± 0.3	8.0 ± 0.2

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

‡ GVHR initiated by i.v. injection of 1.3×10^8 parental strain spleen cells 11 days before infection.

§ Significantly different from control ($P < 0.05$).

contained less than 1%. By 4 hr, virus was undetectable in both organs, but it had reappeared by 10 hr and multiplied progressively over the following 38 hr. Mice with GVHR consistently had lower titers than controls in both liver and spleen, but the most pronounced differences occurred 10 or 24 hr after infection. Mims found that by 10 hr after intravenous injection of ectromelia virus, infected Kuppfer cells transmit infection to adjacent liver parenchyma cells which are not infected directly (8). Thus, since no interferon was detected in the spleens of mice with GVHR, either on the day of infection or 2 days previously, the results in Table III suggested that in these mice, the fixed macrophages of the liver, and presumably the spleen, were more active in phagocytizing and destroying blood-borne virus.

Listeria monocytogenes infection also generates a population of nonspecifically hyperactive macrophages in the livers and spleens of mice (9). Therefore, mice which had been infected with *Listeria* were used in an experiment similar to the preceding one. C57BL mice were given 5 × 10⁴ viable bacteria intravenously. Spleens of four mice were processed for interferon assays on each of days 5 and 7 after infection. On day 7, a group of 20 survivors together with 20 uninfected controls were infected intravenously with 2 × 10⁵ PFU virulent ectromelia virus. Subgroups of four mice were bled after 3 min and their livers and spleens were frozen within 5 min of injection. Other subgroups were sacrificed at 4, 10, 24, and 48 hr and their organs were titrated for virus.

TABLE IV

The Effects of Infection with L. monocytogenes on the Distribution and Behavior of Virulent Ectromelia Virus* after I.V. Injection of 2 × 10⁵ PFU

Time after virus injection	Treatment	Blood	Liver	Spleen
3 min	*L. monocytogenes*‡	2.3 ± 0.2§	—	—
	Control	3.4 ± 0.2	—	—
5 min	*L. monocytogenes*	—	4.7 ± 0.1§	2.5 ± 0.2
	Control	—	5.0 ± 0.1	2.8 ± 0.1
4 hr	*L. monocytogenes*	—	<2.1	<0.7
	Control	—	<2.1	<0.7
10 hr	*L. monocytogenes*	—	2.8 ± 0.4§	2.4 ± 0.3§
	Control	—	4.3 ± 0.1	3.3 ± 0.2
24 hr	*L. monocytogenes*	—	5.7 ± 0.1§	4.5 ± 0.2§
	Control	—	6.2 ± 0.2	5.2 ± 0.3
48 hr	*L. monocytogenes*	—	7.8 ± 0.5	7.9 ± 0.3
	Control	—	8.5 ± 0.5	8.0 ± 0.1

* Expressed as mean log PFU per organ ± standard deviations in groups of four mice.
‡ Infected i.v. with 5 × 10⁴ viable *L. monocytogenes* 7 days before injection of ectromelia virus.
§ Significantly different from control ($P < 0.05$).

The results (Table IV) were similar in many respects to the previous experiment. Infection with *L. monocytogenes* did not cause detectable interferon production in the spleen, but appeared to generate a population of macrophages in the liver and spleen which phagocytized (3 min, Table IV) and destroyed (10 hr, Table IV) ectromelia virus more efficiently than normal.

By taking organs at 5 min, i.e. 2 min earlier than in the preceding experiment and in a similar study with C57BL mice reported earlier (1), a larger proportion (up to 50%) of the virus inoculum could be recovered from the liver. Since Mims (7) has shown that over 95% of ectromelia virus injected intravenously into mice is taken up by Kuppfer cells (with almost 4% implanted in the spleen and less than 1% in the lungs), it seems probable that the failure to detect more of the inoculated virus in the liver in the present experiments was due to rapid uncoating or destruction of virus within littoral macrophages. If it is assumed that up to 95% of injected virus was phagocytized by liver

macrophages, it follows that this population of cells was relatively unproductive, even in normal mice, since 10 hr after infection when the growth cycle in infected littoral macrophages had presumably been largely completed (8), 10% or less of the inoculated dose was present in the liver. Similar reasoning suggests that splenic macrophages were more productively infected than their counterparts in the liver, but they still probably produced little more infectious virus than they ingested (Tables III, IV).

Thus, the macrophages within infectious foci could conceivably play a part in reducing virus titers in these lesions by virtue of the inefficiency with which they are infected by virus they ingest and the small yields of virus from this interaction. Nonspecific hyperactivity would augment their effectiveness in this role.

<div style="text-align:center">DISCUSSION</div>

Histological and immunofluorescence examination of the livers of preinfected recipients of immune spleen cells or hyperimmune serum (Figs. 1–3) showed that the regression of infection was accompanied by the invasion of virus-infected foci by mononuclear cells under immune direction. It was also clear that immune cell transfer promoted denser accumulations of mononuclear cells and more efficient destruction of viral antigens within lesions than did immune serum (Figs. 2, 3). This is in accord with quantitative evidence presented previously (1). Other results reported here (Tables I, II, Figs. 4, 5) suggested that a mononuclear cell invasion of infectious foci triggered by immune mechanisms was also of key importance in recovery from natural primary mousepox. The possible sequence of events in this process and the roles of different factors and cell types will now be considered.

Specifically sensitized lymphocytes are generated by the 4th day of infection (1) and presumably migrate from the circulation into infectious foci. There, upon interaction with viral antigens, a variety of biologically active molecules would be produced (10). At least one of these products, leucotactic factor, attracts mononuclear phagocytes in vitro (11). The activity of such factors would explain the presence within infectious foci of many cells recognizable morphologically as macrophages (Fig. 2). Arguments presented in the preceding paper (1) suggest that these macrophages were derived from blood monocytes.

Even large amounts of antibody were less efficient than specifically sensitized lymphocytes in attracting monocytes to infectious foci in passively immunized mice (Fig. 2). Thus, antibody is probably unimportant in this respect during primary mousepox, since it was not detectable in the circulation before the 7th day (Table II, reference 2).

We can now appraise potentially important antiviral mechanisms which could be active in the mononuclear cell lesions. Firstly, a direct (12, 13) or indirect (14, 15) cytotoxic attack by lymphocytes on virus-infected cells may

occur. Virus-specified antigens are detectable on the surfaces of poxvirus-infected cells in vitro within 2 hr of infection (16). Thus, infected cells could be recognized and attacked before progeny virions are produced within them. Secondly, specifically sensitized lymphocytes may produce interferon upon stimulation with antigen (17). While interferon production was not an important factor in recovery at organ level (Table II), it is possible that specifically induced interferon produced locally could protect cells within and around the lesion as suggested by Glasgow (18). However, in the absence of other mechanisms, both local interferon production and lysis of infected cells by specifically sensitized lymphocytes would logically cause no more than a halt in virus multiplication, since they should not appreciably reduce the numbers of infectious particles already present. Such a halt was observed after transferring immune spleen cells to lethally irradiated recipients in which the antiviral activity could be ascribed mainly to specifically sensitized lymphocytes acting alone (1). More rigidly defined experimental systems may clarify these questions in the future. However, at present it seems likely that the marked fall in virus titers (1) and virtual disappearance of infectious material within lesions (Fig. 3) after immune cell transfer was caused by phagocytosis and intracellular digestion of infectious material by macrophages.

In this respect, the efficiency of macrophages within infectious foci cannot be directly measured. However, information of possible relevance has been obtained by studies of the interaction between ectromelia virus and normal mouse macrophages from different sources. For example, Roberts (6) showed that mouse peritoneal macrophages can be infected by virulent ectromelia virus in vitro but the yield of infectious particles was so small that it could not be accurately quantified. Roberts also estimated that in vivo, infected Kuppfer cells produce of the order of 10 infectious particles/infected cell (19). Since each infected cell may have taken up more than a single particle, and since not all macrophages which ingest virus become infected (8), this interaction appeared to be relatively unproductive.

Quantitative evidence on this question was also obtained in the present investigations. After a 10 hr growth cycle in normal Kuppfer cells in vivo, the yield of virus appeared to be no more than 10% of the amount phagocytized by these cells at the initiation of infection. Littoral macrophages in the spleen, while not as unproductive as liver macrophages, probably generated little more infectious virus than they ingested (Tables III, IV).

Thus, if the blood monocytes which apparently enter infectious foci in target organs acquire the antiviral properties of resident, littoral macrophage populations, they could possibly cause some reduction in virus titers by phagocytosis and intracellular digestion; but the marked fall in titers which occurred within 24 hr of immune cell injection into normal recipients (1) would seem to require additional factors.

There is little doubt that antibody would aid macrophages in destroying infectious material, for the experiments of Roberts (6) showed that even small amounts of immune serum prevented the infection of mouse peritoneal macrophages by ectromelia virus in vitro. However, attempts using immunofluorescence techniques to demonstrate mouse immunoglobulins in the infected foci of adoptively immunized mice have been unsuccessful.[3]

Interferon produced within the lesion by specifically sensitized lymphocytes may also make macrophages less susceptible to infection.

Finally, enhancement of nonspecific intracellular destructive capacity of macrophages can be important in resistance to infection with intracellular bacterial parasites (9) and herpes simplex virus (20). In the present experiments nonspecific stimulation of macrophages in the liver and spleen made them significantly more refractory to ectromelia virus infection (Tables III, IV) and probably resulted in more efficient protection of target organs from blood-borne virus during the primary viremia of mousepox (Table II). This effect was presumably due to increased intracellular digestive activity since interferon could not be found in the spleens of the stimulated mice. However, similar nonspecific hyperactivity of macrophages, as measured by resistance to an intracellular bacterial parasite, *Listeria monocytogenes*, was not detectable in the liver and spleen during the recovery process in mousepox.[3] While these results cannot exclude the possibility that macrophage hyperactivity was present, but limited to the infectious foci, they make it seem less likely that such activity plays an important role.

SUMMARY

Histological and immunofluorescence techniques showed that mononuclear cells invaded virus-infected foci in the livers of passively immunized mice within 10 hr of the receipt of immune spleen cells or hyperimmune serum; by 24 hr, marked destruction of virus antigens had occurred in these lesions. Immune cell transfer promoted denser packing of mononuclear cells in the foci and more efficient destruction of infectious material than immune serum. Similar liver lesions developed by the 6th day after sublethal, primary, subcutaneous infection in normal mice. In contrast, in mice with GVHR which were immunosuppressed but possessed hyperactive macrophages and unimpaired splenic interferon response, mononuclear cells did not invade liver lesions and the animals died. These results, together with data reported previously, indicated that mononuclear cell invasion of infected liver foci, triggered by CMI, was of key importance in recovery from primary mousepox.

The roles of specifically sensitized lymphocytes and macrophages within lesions were not directly evaluated, but indirect evidence suggested that lymphocytes could cause no more than a halt in virus multiplication, and that

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[3] Blanden, R. V. 1970. Unpublished experiments.

macrophages were required for the inactivation of preformed virions. Possible augmentation of the efficiency of macrophages by locally-produced lymphocyte interferon, neutralizing antibody, or stimulation of their phagocytic and intracellular digestive capacity cannot be excluded.

BIBLIOGRAPHY

1. Blanden, R. V. 1971. Mechanisms of recovery from a generalized viral infection: mousepox. II. Passive transfer of recovery mechanisms with immune lymphoid cells. *J. Exp. Med.* **133**:1074.
2. Blanden, R. V. 1970. Mechanisms of recovery from a generalized viral infection: mousepox. I. The effects of anti-thymocyte serum. *J. Exp. Med.* **132**:1035.
3. Blanden, R. V. 1969. Increased antibacterial resistance and immunodepression during graft-versus-host reactions in mice. *Transplantation.* **7**:484.
4. North, R. J. 1970. The relative importance of blood monocytes and fixed macrophages to the expression of cell-mediated immunity to infection. *J. Exp. Med.* **132**:521.
5. Fenner, F. 1949. Mousepox (infectious ectromelia of mice): a review. *J. Immunol.* **63**:341.
6. Roberts, J. A. 1964. Growth of virulent and attenuated ectromelia virus in cultured macrophages from normal and ectromelia-immune mice. *J. Immunol.* **92**:837.
7. Mims, C. A. 1959. The response of mice to large intravenous injections of ectromelia virus. I. The fate of injected virus. *Brit. J. Exp. Pathol.* **40**:533.
8. Mims, C. A. 1959. The response of mice to large intravenous injections of ectromelia virus. II. The growth of virus in the liver. *Brit. J. Exp. Pathol.* **40**:543.
9. Mackaness, G. B., and R. V. Blanden. 1967. Cellular immunity. *Progr. Allergy.* **11**:89.
10. Lawrence, H. S., and M. Landy, editors. 1969. Mediators of cellular immunity. Academic Press, Inc., New York.
11. Ward, P. A., H. G. Remold, and J. R. David. 1969. Leucotactic factor produced by sensitized lymphocytes. *Science (Washington).* **163**:1079.
12. Perlmann, P., and G. Holm. 1969. Cytotoxic effects of lymphoid cells *in vitro.* *Advan. Immunol.* **11**:117.
13. Brunner, K. T., J. Mauel, H. Rudolf, and B. Chapuis. 1969. Homograft immunity: mechanisms of immunologic enhancement and the cellular immune reaction *in vitro. In* Cellular Recognition. R. T. Smith and R. A. Good, editors. Appleton-Century-Crofts, Inc., New York. 243.
14. Ruddle, N. H., and B. H. Waksman. 1967. Cytotoxic effect of lymphocyte antigen interaction in delayed hypersensitivity. *Science (Washington).* **157**:1060.
15. Granger, G. A., S. J. Shacks, T. W. Williams, and W. P. Kolb. 1969. Lymphocyte *in vitro* cytotoxicity. Specific release of lymphotoxin-like materials from tuberculin-sensitive lymphoid cells. *Nature (London).* **221**:1155.
16. Ueda, Y., M. Ito, and I. Tagaya. 1969. A specific surface antigen induced by poxvirus. *Virology.* **38**:180.

17. Green, J. A., S. R. Cooperband, and S. Kibrick. 1969. Immune specific induction of interferon production in cultures of human blood lymphocytes. *Science (Washington)*. **164:**1415.

18. Glasgow, L. A. 1970. Cellular immunity in host resistance to viral infections. *Arch. Intern. Med.* **126:**125.

19. Roberts, J. A. 1963. Histopathogenesis of mousepox. III. Ectromelia virulence. *Brit. J. Exp. Pathol.* **44:**42.

20. Hirsch, M. S., B. Zisman, and A. C. Allison. 1970. Macrophages and age-dependent resistance to herpes simplex virus in mice. *J. Immunol.* **104:**1160.