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Mechanisms Determining Innate Resistance to Ectromelia Virus Infection in C57BL Mice

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The mechanism for innate resistance to ectromelia virus, which is controlled by a single gene in C57BL mice, was investigated. The cells or factors involved appear to be radioresistant and to impose an early barrier to viral penetration and spread via lymphatics or blood to the target organs, i.e., liver and spleen.

A previous study of mortality in inbred mouse strains infected with ectromelia virus has revealed two distinct genetic elements in resistance (unpublished data). A single, dominant autosomal gene in C57BL mice (B6 and B10 strains) confers innate resistance and gives a 50% lethal dose [LD_{50}] between 10^2 and 10^7 PFU higher than in other mouse strains. Several other genes which affect resistance have been mapped to the *H-2* gene complex, the major histocompatibility complex in the mouse, but they are expressed only in mice carrying the C57BL background gene (H. C. O'Neill, R. V. Blanden, and T. J. O'Neill, Immunogenetics, in press).

The pathogenesis of ectromelia infection in susceptible mice was originally documented by Fenner (6, 7). After footpad inoculation of the virulent Moscow strain of virus, infection rapidly spreads into the lymphatics and through the bloodstream to the target organs, i.e., the spleen and liver. In susceptible strains, death occurs rapidly from 5 days after infection before any immune response is detectable and is associated with massive liver necrosis.

More recent studies in resistant C57BL mice have indicated the importance of a cell-mediated immune response in recovery from infection (3–5). A role for both T cells with cytotoxic activity (Tc cells) and mononuclear phagocytes has been demonstrated (3, 11), and both cell types are distributed via blood to sites of infection. Tc cells can recognize virus-induced changes in infected-cell surfaces as early as 1 h after infection (1) and would appear to limit the spread of infection by lysing these cells before viral replication ensues. Blood monocytes enter sites of infection as a result of T cell action and ingest free virus and cell debris (5). Both mononuclear phagocytes and Tc cells can become infected in such sites and may be destroyed by other Tc cells, but both can be continually replenished via the blood. Recovery is therefore a dynamic process, involving a race between onset of the Tc cell response and invasion of virus into the lymphoreticular system.

The mechanism determining innate resistance in C57BL mice is unknown but appears to act at an early stage after infection since susceptible strains show affected behavior by day 3 or 4 and die from day 5 after infection. The mechanism appears to be unrelated to immune response capacity since death or survival is determined before a Tc cell response can be detected. For some viral infections where strain differences in macrophage susceptibility have been reported (9, 13, 14), innate resistance has been correlated with the inability of mononuclear phagocytes to support virus growth and to act as an early barrier to virus spread. However, similar studies with ectromelia virus (16; unpublished data) have indicated no major differences in viral recovery from cultures of infected macrophages and normal or immune spleen cells.

Therefore, the experiments reported here were designed to investigate the early barrier to infection, which confers innate resistance to ectromelia virus in C57BL mice, and to make a comparison with other *H-2*-linked mechanisms of resistance. Virus trace experiments were performed in C57BL/6J (resistant) and BALB/c (susceptible) mice to compare early virus spread into the lymphatic system and through blood to the spleen and liver (for genotype and LD_{50} data, see Table 1).

Age- and sex-matched mice were inoculated in the hind footpad with 10^5 PFU of virulent Moscow strain ectromelia virus, and groups of four mice were sacrificed daily for virus titration in the draining popliteal and iliac lymph nodes, the target organs, i.e., liver and spleen, as well as in blood and inguinal lymph nodes (Table 2). Mortality tests were performed on a control group of mice to confirm the virulence of the

TABLE 1. Mouse strains used in this study

Strain	*H-2* haplotype[a]								LD_{50}[b]	Resistance[c]
	K	*I-A*	*I-B*	*I-J*	*I-E*	*I-C*	*S*	*D*		
C57BL/6J, C57BL/10J(B10)	b	b	b	b	b	b	b	b	10^5	R
BALB/c	d	d	d	d	d	d	d	d	3.0	S
B10.A	k	k	k	k	k	d	d	d	3.1×10^3	SR
A/J	k	k	k	k	k	d	d	d	0.04	S

[a] Haplotype origin of strains according to Klein et al. (12).
[b] Assay performed with virulent Moscow strain ectromelia virus, subcutaneous inoculum into footpad. Data extracted from Blanden et al. (submitted for publication).
[c] Mouse strains were classified as either resistant (R), susceptible (S), or semi-resistant (SR).

virus stock. All BALB/c mice were dead by day 6, so that virus titers are available for the BALB/c strain only for the first 5 days after infection. Throughout this time frame, in all tissues examined, BALB/c mice generally exhibited earlier detectable infection and higher virus titers than C57BL/6J mice (Table 2). Most importantly, although infection was first detected in the spleens and livers of both strains on day 3, titers in BALB/c mice were always 1 to 2 $\log_{10}$ higher than in C57BL/6J mice. The more rapid dissemination of infection in BALB/c mice was also reflected in their earlier detectable viremia, although infection of the liver and spleen must have occurred via the blood in both strains.

Although differences in the levels of virus first reaching the spleen were highly significant, virus appeared to multiply at the same rate in each strain until death in BALB/c mice or until day 5 or 6 in C57BL/6J mice, when spleen virus titers were reduced significantly. Another point worthy of comment is the delay of 3 days in the time of virus transmission from the popliteal to the iliac node in C57BL/6J mice compared with a delay of 1 day in BALB/c mice.

Since BALB/c mice had consistently higher virus titers in lymphoid tissues than C57BL/6J

TABLE 2. Ectromelia virus growth in resistant and susceptible mouse strains

Organ[a]	Strain[b]	$\log_{10}$ virus titer on postinfection day[c]:							
		1	2	3	4	5	6	7	8
Popliteal lymph node	C57BL/6J	0.4	3.7 ± 0.1^1	3.6	4.7 ± 0.4^2	5.8 ± 0.1	3.9 ± 0.5	3.7 ± 0.4	4.3 ± 0.4
	BALB/c	1.5	4.4 ± 0.2^1	5.8	5.6 ± 0^2	5.5 ± 0			
Iliac lymph node	C57BL/6J	<0.4	<1.6	<1.6	<1.6	3.5 ± 0.5	2.5 ± 0.6	1.7 ± 0.1	2.2 ± 0.4
	BALB/c	<0.4	<1.6	4.00	4.4 ± 0.4	4.9 ± 0.3			
Inguinal lymph node	C57BL/6J	<0.4	<1.6	1.6	<1.6	<1.6	<1.6	1.6	1.7 ± 0.1
	BALB/c	<0.4	<1.6	2.7	3.1 ± 0.4	3.6 ± 0.2			
Blood cells[d]	C57BL/6J	<0.7	<0.7	<0.7	<0.7	0.7	0.7	1.5	0.7
	BALB/c	<0.7	<0.7	0.7	1.0	2.6			
Spleen	C57BL/6J		<1.4	2.9 ± 0.3^3	3.4 ± 0.2	5.4 ± 0.5	3.5 ± 0.1	4.0 ± 1.0	4.2 ± 0.2
	BALB/c		<1.4	4.9 ± 0.3^3	5.6 ± 0.2	6.7 ± 0.2			
Liver	C57BL/6J	<2.4	<2.4	2.6 ± 0.1^4	3.2 ± 0.4	4.7 ± 0.2	4.3 ± 0.6	3.7 ± 0.5	4.3 ± 0.4
	BALB/c	<2.4	<2.4	3.8 ± 0.1^4	4.7 ± 0.5	5.6 ± 0.2			

[a] Whole organs were snap frozen in an ethanol-dry ice bath and then stored at −70°C. Before virus titration, tissues were thawed, ground in Puck's saline, sonicated, and centrifuged to remove cell debris.
[b] Eight-week old, female mice were inoculated subcutaneously into the left hind footpad with 10^5 PFU of virulent Moscow strain ectromelia virus on the same day, using a micrometer syringe delivering 10-μl volumes. Four animals were then sacrificed daily for assay until day 5 for BALB/c mice and until day 8 in C57BL/6J mice. A concurrent analysis in mice given 10^2 PFU proved to be an equivalent study. Mortality tests were performed on control mice to confirm virulence of virus.
[c] Values represent mean ± standard error ($n = 4$), unless one titration was performed on a pool from four animals. Virus titers were determined by a plaque assay on L929 cells (3). Data points with the same superscript number are significantly different ($P < 0.05$) by Student's t test.
[d] Values represent the titer of virus per milliliter of whole blood from a pooled example.

mice, even as early as 1 day after infection in the popliteal lymph node draining the inoculation site, chimeric mice were used to determine whether the C57BL background gene responsible for innate resistance expressed this function through radiosensitive lymphomyeloid cell populations. Mice of *H-2*-compatible strains B10.A (resistant) and A/J (susceptible) (see Table 1 for LD_{50} data) were lethally irradiated (950 rad), reconstituted with fetal liver stem cells from either B10.A or A/J donors, and infected 15 weeks later in the foot with various doses of virulent ectromelia virus (Table 3). Since the production of chimeras is logistically demanding and numbers were not available for doing LD_{50} and virus trace experiments, the simpler indicator of time to death was used. A clear difference of 2 days in the average day of death was found, which was related to the genotype of the irradiated host and not to the stem cell donor. The overall mortality and time to death in chimeras was clearly determined by the genotype of the irradiated host and not by the stem cell donor. This indicates that the effect of the C57BL background gene which confers resistance is not expressed through radiosensitive lymphomyeloid cells (e.g., stem cells, T cells, or B cells) or through tissue macrophages, which would have been largely replaced by cells of donor origin in the 15 weeks after reconstitution (2, 8, 10, 18), but acts through radioresistant cells or mechanisms. In contrast, other chimera experiments have shown that *H-2*-linked genes influence resistance through radiosensitive lymphomyeloid cells (O'Neill et al., in press).

TABLE 3. Resistance of *H-2*-compatible irradiation chimeras to ectromelia virus infection

Strain[a]	Day of death ± SE at a virus dose (PFU) of[b]:	
	10^3	10^2
B10.A control	9.2 ± 0.7[1]	8.0 ± 0.0 (50)
A/J → B10.A[c]	9.3 ± 0.3[2]	9.2 ± 0.7[3]
A/J control	7.2 ± 0.2[1]	7.0 ± 0.0
B10.A → A/J	7.0 ± 0.0[2]	7.3 ± 0.3[3]
B10.A → B10.A	ND[d]	8.4 ± 0.3[4]
A/J → A/J	ND	6.5 ± 0.2[4]

[a] Groups of between four and six mice were inoculated into the footpad with Moscow strain ectromelia virus and deaths were scored daily for 14 days.

[b] Mortality was 100% in all cases, except where the actual percent mortality is entered in parentheses. Data points with the same superscript number are significantly different ($P \leq 0.05$) by Student's t test.

[c] Chimeras were prepared by irradiation (950 rad) of adult (8 week) mice and reconstitution with 2×10^7 4×10^7 fetal liver stem cells from 16-day embryos given intravenously. Mice were used at 15 weeks postreconstitution.

[d] ND, Not determined.

The data presented indicate that the superior resistance of C57BL/6J over BALB/c operates within 1 or 2 days after infection through reduced virus transmission into the lymphoreticular system. This effect seems to operate through cells or mechanisms located in or between the skin of the foot and the popliteal lymph node. Even though great care was taken to minimize foot tissue damage during injection, the possibility remains that foot tissue and small vessels may have become disrupted so that some virus could directly enter the lymphatics and blood, leading to an early pulse of virus into the popliteal node and other target organs. In the absence of such mechanical damage, one might expect to see an even greater delay in the transmission of virus from the foot to the draining lymph nodes. Support for such a proposal is shown by the noticeable delay in virus transmission between popliteal and iliac node in C57BL/6J mice, which would appear to occur too late to determine innate resistance but which must also reflect the fact that virus transmission through the lymphatic system is slower in C57BL/6J than in BALB/c mice.

With respect to defining cells or factors which determine innate resistance, the work of Roberts (15) showed that initial ectromelia infection in the foot progressed more rapidly in the dermis than in the epidermis, so epidermal cells are not strong candidates. Furthermore, since Schell (17) has shown that virulent ectromelia virus grows at the same rate in the feet of C57BL/6J and susceptible outbred mice, fibroblasts are unlikely to be important in the genetic difference observed here. Therefore, radioresistant cells or factors which might influence the rate of viral penetration and spread via lymphatics or blood vessels would seem to be prime candidates for further study.

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