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Role of Macrophages in Natural Resistance to Virus Infections

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INTRODUCTION

In analyzing host defense mechanisms against virus infections, two aspects have to be considered. The first aspect concerns the mechanisms by which the host displays resistance to and recovers from viral infections encountered for the first time in life; the second concerns the defense mechanisms mounted by an immune host to reinfection. In both instances, the principal tasks of host defenses are to prevent establishment of infection and, if infection has happened, to interfere with virus multiplication, limit the spread of infection, and finally eliminate the virus. To achieve this, a complex interaction of both specific and nonspecific host defense mechanisms has to be at work.

In primary infections, nonspecific host resistance factors represent the main line of defense during the first few days of infection. These consist of preexisting defense mechanisms, such as barriers to virus penetration (cutaneous epithelium, ciliated respiratory epithelium, vascular endothelium, lack of membrane surface receptors, etc. [94]), nonspecific viricidins in body fluids (138), and phagocytosis leading to virus destruction (98). A further series of nonspecific antiviral principles are induced fairly rapidly. These are factors like interferon (15, 67), elevated body temperature (86), and acid pH and hypoxia in inflammatory exudates (14). By interfering with the early stages of virus invasion, multiplication, and spread to susceptible organs, the nonspecific host defenses may be important in determining the outcome of a virus infection. Final recovery from a fully established infection, however, is probably determined by the specific immune response, appearing some days after the initiation of infection.

In addition to being of importance in recovery from fully established primary infections, specific host responses are the main defense mechanisms against reinfection with the same or antigenically closely related organisms. They are mounted by cells of the lymphoid system and

involve the production by the B-lymphocyte system of antibodies with the capacity to neutralize extracellular virus and cellular immunity mediated by sensitized T lymphocytes. Being immunological in nature, they are specifically induced and act specifically against the virus concerned. An intense interaction between B- and T-cell-mediated immune responses to virus infections occurs, and other factors, such as complement and cells from the mononuclear phagocyte system (macrophages) are involved as helpers or mediators in the immune response (3).

The objective of this review was to analyze the role played by macrophages in natural resistance to primary virus infections. Special emphasis is placed on the key role of macrophages as a first line of defense at the portal of entry or in target organs of crucial importance for the outcome of an infection. The review does not emphasize immunological aspects of virus-macrophage interactions; information on this topic can be found in other reviews (3, 23).

THE MONONUCLEAR PHAGOCYTE SYSTEM

The term *macrophage* ("large eater") was introduced by Metchnikoff in 1892 (95). He classified phagocytic cells as "macrophages" and "microphages" (polymorphonuclear leukocytes) on morphological and functional grounds. He demonstrated that these cells play an important role in resistance to bacterial infections by engulfment and digestion of the invading microorganisms. Mononuclear phagocytes resident in various organs and tissues were, according to Metchnikoff, closely related and belonged to a "macrophage system."

This system was further elaborated by Aschoff (8), who grouped reticular cells of the spleen and lymph nodes and reticuloendothelial cells of the lymph and blood sinuses as well as histiocytes, splenocytes, and blood monocytes in a unifying cell system called the "reticuloendothelial system." Despite much criticism (92) and suggestions of other terms, such as the "reticulohistiocyte system" (144, 156), the concept of Aschoff's reticuloendothelial system has been widely accepted and used until today. However, our current knowledge of phagocytic mononuclear cells recently demanded a reevaluation of the classification of these cells. On the basis of morphology, function, and kinetics of development, all highly phagocytic mononuclear cells and their precursors can fit into one cell lineage called the "mononuclear phagocyte system" (154).

The morphology of cells of the mononuclear phagocyte system depends to certain degrees on the species of animal, the anatomical site, and the degree of development or stimulation (60). Certain common characteristics can, however, be outlined. They are fairly large cells with varying amounts of cytoplasm and a reniform or oval nucleus. Cytoplasmic organelles for secretory activities are abundant in the more mature forms present in the tissues (154). Ruffling of the surface membrane, easily recognized in phase-contrast or electron microscopy, is a specific morphological characteristic of the mononuclear phagocytes as compared with lymphocytes (60).

The functional properties shared by cells of the mononuclear phagocyte system are avid phagocytosis, pinocytosis, and the ability to adhere firmly to a glass or plastic surface (154). Glass adherence is a major means of isolating the cells. Other cell types (for example, fibroblasts, reticular cells, and endothelial cells) can also phagocytize foreign particles, but to a much lower degree (116). Furthermore, the ingestion of particles by mononuclear phagocytes is enhanced by the presence of specific immunoglobulins with or without complement ("immune phagocytosis"), because these cells have Fc (18) and complement (78) receptor sites at the cell membrane. This is probably not the case with the other cell types.

Cytokinetic studies have shown that the cells of the mononuclear phagocyte system and their precursors represent a single cell lineage (153). Mononuclear phagocytes originate from precursor cells of the bone marrow; here, these cells mature into promonocytes and monocytes. Monocytes are transported via the blood to organs and tissues, where they finally mature into macrophages.These are histiocytes of connective tissue; Kupffer cells of the liver; alveolar macrophages of the lungs; free and fixed macrophages of the spleen, lymph nodes, and bone marrow; and macrophages monitoring serous spaces, such as the pleural and peritoneal cavities. Osteoclasts of bone tissue and microglial cells of the nervous system have more tentatively been included in the system (154). Blood monocytes continuously "feed" these compartments with new cells. In sites of inflammation, the recruitment of monocytes from the blood is greatly accelerated, although local multiplication of mature macrophages apparently may also occur (111).

ASSESSMENT OF VIRUS-MACROPHAGE INTERACTIONS IN VITRO

Sources of Macrophages

Macrophage cultures are easily started and can be maintained in vitro for weeks (68); in fact,

macrophages were one of the first cell types to be established in culture (27). In mammals and birds, cells can be obtained from serous cavities or various organs. The choice of a macrophage donor depends on the particular virus-host interaction under study and the cell number required for the investigations. For many purposes, the mouse is a convenient experimental animal, because different inbred and congenic strains are available. From the peritoneal cavities of mice as well as rats, rabbits, and guinea pigs, resident macrophages can be harvested by simple lavage of the cavities with buffered saline or culture medium (31, 36). To increase the yield of cells, various inflammatory agents, such as mineral oil, glycogen, proteose-peptone, etc., are often used to induce an exudate in the peritoneal cavity. However, in addition to increasing the yield, individual cells harvested will be in a state of activation and may not reflect the properties of native, uninduced cells (36). Other sources of macrophages have been used. Large numbers of alveolar macrophages can be obtained by lavage of the lower respiratory tracts of experimental animals (108). Bovine macrophages in enormous quantities can be harvested from the mammary gland after infusion of lipopolysaccharide in the gland (157). By explanting fragments of livers from newborn mice onto a reconstituted collagen substrate (37) in a medium supplemented with horse serum and chicken embryo extract, Bang and Warwick (13) obtained migration of liver macrophages away from the explants. Recently, a technique has been described by which liver macrophages can be isolated by perfusion and digestion with Pronase (38).

In humans, the only readily accessible source of cells of the mononuclear phagocyte system is monocytes from peripheral blood. Several methods for the isolation of these cells have been devised, including gradient centrifugation on dense albumin (17) or Ficoll-Hypaque (24) solutions combined with a glass adherence step. After 2 or 3 days in culture, the cells show macrophage characteristics.

In Vitro Studies on Virus-Macrophage Interactions

The ability of virus particles to grow in macrophages can be a major pathogenicity or virulence factor in virus diseases, because macrophages monitor the main body compartments and thereby may determine the access of virus particles to susceptible tissues or organs (98). Several in vitro methods have been used in the study of virus particle uptake into and replication in macrophages. Although the outcomes of virus-macrophage interactions as regards these parameters have been elucidated for many viruses, little is still known about the intracellular events which enable a virus to replicate in the macrophage instead of being digested or what factors determine the failure of macrophages to digest the virus particle, making a replication possible (99). Because the yields of infectious virus released from infected macrophages are very low for many viruses as compared with the yields from other cell types, indirect assays are often used to estimate the ability of a virus to replicate in these cells.

Adsorption. The first step in virus-macrophage interaction is adsorption of the virus particle to the cell. Adsorption experiments can be conducted with both monolayer and suspension cultures of macrophages. Two different approaches to the assessment of adsorption have been applied. The first uses measurement of the fractions of residual, unadsorbed virus in the culture medium after different times of adsorption (101, 135, 141). Appropriate controls without macrophages have to be included to take account of heat inactivation and adherence of virus to the glass or plastic surface. By the second method, the amount of intracellular virus is determined after various times of adsorption and thorough washing of the cultures (62, 146). This method misses the eclipsed virus and thus does not accurately reflect the total amount of adsorbed virus. The information gained of differences in virus particles replication in various virus-macrophage systems is only valid if the adsorption rates are equal.

Direct assay of virus growth. In some viruses, it has been possible to measure directly virus growth in cultured macrophages by simple titration of extracellular virus released into the culture medium or of intracellular virus released from the cells by freezing and thawing or ultrasonic vibration. In one of the most productive systems studied, Shif and Bang (135) were able to grow mouse hepatitis virus type 2 (MHV-2) in cultures of peritoneal macrophages from susceptible Princeton (PRI) mice to titers close to 10^8 macrophage tissue culture mean infective doses per ml. The replication of MHV-3 in macrophages has also been demonstrated by a simple titration approach (155). Lactic dehydrogenase virus replicates mainly, or perhaps exclusively, in mouse macrophages (35) and grows to high titers in cultures of these cells (34). Lindenmann et al. (82) adapted a strain of avian influenza A virus to grow to high titers in mouse macrophage cultures as assessed by virus titration and hemagglutinin measurement. Less efficient is the replication of flaviviruses in mouse macrophages, but direct titrations of West Nile virus and yellow fever virus yields from mouse

macrophage cultures prepared from resistant and susceptible strains of mice have been sensitive enough to reveal the importance of macrophages in determining resistance to these viruses (48, 49). Growth of vaccinia virus in macrophage cultures from mice (118) and rabbits (131) has been used to evaluate the role of activated and immune macrophages in host defenses. For herpes simplex virus type 1 (HSV-1) (69, 141) and murine cytomegalovirus (132), the yields have generally been less than 10% of the initial inocula.

Indirect assays of virus growth. Indirect assay methods have been used to measure virus growth in virus-macrophage systems in which the yields are too small for direct titration. Furthermore, these techniques (i.e., immunofluorescence, electron microscopy, and autoradiography) have been used in attempts to disclose the intracellular events occurring in virus-infected macrophages.

(i) Infectious center assays. In infectious center assays, the infected macrophages are cocultivated with a permissive cell monolayer. Two major modifications of the technique have been applied. Either macrophages are grown on a glass or plastic surface, infected with the virus, and finally overlaid with a monolayer of susceptible target cells (62, 69, 101, 146), or a suspension of infected macrophages is added to a preformed permissive monolayer (117, 132, 150). The release of infectious virus from the macrophages can then be estimated by counting the number of infectious centers appearing in the indicator cell monolayer either as plaques (62) or as fluorescent foci (69). It is important that all nonadsorbed virus be eliminated before cocultivation; this is usually achieved by washing the macrophages with antiserum. Another imperative demand in these assays is to work with pure macrophage populations without contamination with permissive cells, such as fibroblasts. The infectious center assays indicate the number of macrophages in the culture that support virus replication, but they do not give any information of how well the individual cells do so.

(ii) Cytopathology. Direct observation of cytopathic changes of virus-infected macrophages has not been widely used to evaluate virus-macrophage interactions because cellular changes are not very prominent with many viruses studied. However, MHV-2 causes such marked degenerative changes in macrophages from susceptible mice that this effect has been used as the main marker for susceptibility or resistance of whole animals to the virus (13, 71), and it has been possible to develop a plaque assay for this virus in macrophage monolayers (134). Similarly, MHV-3 induces another easily detectable cytopathic effect in macrophages from susceptible mice with the formation of multinucleated giant cells (90, 155). A macrophage-adapted strain of avian influenza virus induces rounding and clumping of macrophages from susceptible mice with a characteristic blurring of the outlines of the cells (82). Occasional production of polykaryocytes by HSV-1 in mouse macrophage cultures was described by Stevens and Cook (141).

(iii) Immunofluorescence. Immunofluorescent visualization of virus antigen in infected macrophage cultures has proven useful in studying both quantitative and qualitative aspects of virus-macrophage interactions. Both direct and indirect techniques have been used. By these methods, information has been gained of the actual numbers of macrophages being infected with West Nile virus (49, 150), ectromelia virus (122), HSV (69, 141), lymphocytic choriomeningitis virus (145), and MHV (155). Furthermore, the time courses of intracellular development of virus antigen have been followed in macrophage cultures infected with HSV (69) and ectromelia virus (122), and the transmissions of newly synthesized HSV (69) and MHV (152) to surrounding macrophages have been monitored. Specific results obtained by using the immunofluorescence technique in various virus-macrophage systems are given in more detail in later sections of the review.

(iv) Electron microscopy. Although electron microscopy should offer a good means of studying the morphogenesis of virus particles in macrophages, it has only been applied in a few studies. Johnson (69) found that suckling mouse macrophages infected by HSV-1 contained large numbers of apparently normal virus particles in the cytoplasm. Stevens and Cook (141), on the other hand, found in their study of adult mouse macrophages that only a few morphologically complete HSV particles were seen in the cytoplasm of infected macrophages, whereas most cells contained empty capsids lacking a dense central core. Other models with similar age-dependent restrictions of virus growth or models with genetically determined or virus strain-related restrictions could probably take advantage of the technique in a search for the mechanisms by which virus replication is restricted in macrophages.

(v) Tracer techniques. Macrophages do not divide in vitro (16), and deoxyribonucleic acid (DNA) synthesis in macrophages infected with DNA viruses can generally be regarded as virus specific. Macrophages labeled with [^{3}H]thymidine during infection incorporate the radioactive

material into nascent virus-specified nucleic acid, which can then be assayed by counting disintegrations in extracted DNA (40, 141). The viral origin of the DNA can be verified by buoyant density determination after equilibrium sedimentation in CsCl gradients (141) or by comparisons of reassociation kinetics (40). The proportion of infected macrophages producing viral DNA may be determined by autoradiography (69, 141). Virus-induced protein synthesis in infected macrophages can be detected by labeling the infected cells with [^{3}H]thymidine and ^{14}C-labeled reconstituted protein hydrolysates and identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (141).

ASSESSMENT OF VIRUS-MACROPHAGE INTERACTIONS IN VIVO

In vivo studies are as essential for the understanding of the participation of macrophages in the pathogenic events during virus infections as are in vitro investigations. First, a complexity of virus-host interactions is involved in in vivo infection as compared with the much simpler and more readily controllable virus-cell interaction in tissue culture. Second, during collection and cultivation, macrophages might lose their native properties, or new ones might be gained in vitro. The latter point is of special importance when the cells are induced by inoculation of an inflammatory agent before collection to increase the yield. These stimulated macrophages are qualitatively different from the cells normally resident in the animal. Furthermore, cells collected after injection of different compounds may differ in a number of characteristics (36), for example, in their ability to support virus replication (152).

General Markers of Pathogenicity

In most studies on the role of macrophages in host defenses against virus infections, general markers of pathogenicity are used to evaluate the outcomes of virus-macrophage interactions and the effects of various modulations of the mononuclear phagocyte system.

Morbidity and mortality. Data on disease and death are perhaps the parameters most commonly used in the study of virus-host interactions. As integrated parts of other in vivo and in vitro studies, they represent valuable markers for pathogenicity or virulence of the virus or for susceptibility or resistance of the host. Specific signs of virus-induced disease, for example, pneumonia, hepatitis, and encephalitis, may, furthermore, reveal the target organ for the infection. However, they provide little information on the site of initiation of infection, the spread of infection through the body, the cells supporting virus growth, and the antiviral host defense mechanisms that are counteracting the infection.

Virus clearance and virus growth. Assays of organs and tissue suspensions for virus at different times after infection reveal, like morbidity and mortality, only gross features of the pathogenesis of a virus infection. Clearance curves for the disappearance of inoculated virus and growth curves for virus in the main target organs and tissues may be constructed, but, taken alone, no information is gained on the types of cells that are infected and the factors that are acting against the infectious process. But, again, infectivity titrations analyzed in the light of information obtained by other means are often valuable or even necessary for interpretation of the accumulated data on the pathogenic events.

Histological lesions. Examination of sectioned organs and tissues taken from animals during infection has been extensively used in the study of the pathogenesis of virus diseases. By routine histological examinations, the development of pathological lesions in the main target organs can be followed, and the cells undergoing degeneration or developing inclusion bodies can be studied. Most often, the affected areas are infiltrated with inflammatory cells, such as polymorphonuclear leukocytes, lymphocytes, and mononuclear phagocytes, and an estimation of the time course of their appearance and their relative number in relation to, for example, changes in the amount of virus recovered from the organ may give information on their participation in host defenses against the infectious process.

By the fluorescent antibody technique, infected cells can be readily identified. The primary uptake of the virus by macrophages in tissues and organs can be studied, and the intracellular fate of the virus particles can be followed (fading or increasing brightness of fluorescence). Eventual spreading of the infection to surrounding parenchymal cells can be monitored. Extensive application of this technique has been of the utmost importance for the elucidation of the role of macrophages in the pathogenesis of ectromelia virus infection in mice, which thereby has become one of the best studied models of generalized viral infection (22, 98, 121).

Modulations of the Function of the Mononuclear Phagocyte System

Macrophage blockade. One way of delineating the role of mononuclear phagocytes in host

resistance to virus infections in vivo is to selectively inhibit the macrophage population of the animal. This can be done by treating the animal by either specific antimacrophage serum or certain chemical agents. Although crude antimacrophage serum was prepared as early as 1899 by Metchnikoff (96), antiserum reacting specifically with macrophages was first produced by Cayeux et al. (28) and Unanue (147), by immunizing rabbits with cultured mouse peritoneal macrophages. The first attempts to modify the course of virus infection by antimacrophage serum were made in mice by Panijel and Cayeux (115), with equivocal results: increasing susceptibility to yellow fever virus infection contrasting with an apparent protection against encephalomyocarditis virus infection. Hirsch et al. (61) achieved some increase in the susceptibility of baby mice to vesicular stomatitis virus infection by inoculation of antimacrophage serum intraperitoneally at daily intervals for 3 days. The resistance of adult mice to infection with HSV (167) and rabies virus (146) was also significantly reduced by intraperitoneal inoculation of antimacrophage serum for 3 days.

Chemically induced macrophage blockade has been more widely applied. Several compounds have been used, but among these silica (silicon dioxide) seems second to none as regards potency and specificity (4). It has been shown that silica is selectively toxic to macrophages (5, 73, 114). In mice, intraperitoneal doses of 50 mg and intravenous doses of 3 mg are well tolerated, and such doses have been used to examine the role of macrophages in infections with HSVs (34, 104, 167), yellow fever virus (168), Coxsackie B-3 virus (117), murine cytomegalovirus (132), Friend leukemia virus (75, 163), rabies virus (146), lactic dehydrogenase virus (34), influenza virus (55), and Semliki Forest virus and encephalomyocarditis virus (4). In most studies, a German sample of silica, Dörentrup no. 12, <5 μm in diameter, distributed by A. C. Allison, Clinical Research Centre, Harrow, England, has been used, which makes possible a comparison of the results obtained with different viruses.

Macrophage stimulation. Another way of assessing the potentials of macrophages to limit virus spread in vivo uses the administration before or during infection of various immunomodulators which are known to elicit augmented recruitment to tissues and organs of hyperactive mononuclear phagocytes from the bone marrow and blood. If such treatments result in increased resistance to the virus under study, the mononuclear phagocyte system may also be assigned a role in the natural defenses against the virus. Both live microorganisms, such as attenuated *Mycobacterium bovis* (Bacille Calmette-Guérin [BCG]) (9, 76, 140) and *Staphylococcus aureus* (133), and nonviable microbial preparations, such as staphylococcal phage lysate (133, 140) and typhoid and *Brucella* vaccines (140), have been used, as well as synthetic compounds, such as levamisole, an anthelmintic drug (140).

Macrophage transfer. Since a successful transfer of cells can be achieved only in syngeneic animals, most macrophage transfer studies have been conducted in mice, where macrophages have been transferred from inbred adult mice to usually more susceptible young or suckling mice of the same inbred strain. Protection of the recipient mice against subsequent infection can then be ascribed to the transferred cells. This approach has been used to analyze age-related resistances of mice to infections with HSVs (62, 69, 103), murine cytomegalovirus (132), Coxsackie B-3 virus (117), and rabies virus (146). Usually, a total of 10^6 to 10^7 cells are given by the intraperitoneal route immediately before infection, but transfer of macrophages from adult mice to 3-week-old mice by intravenous inoculation has also been applied (103).

ASPECTS OF MACROPHAGE-DEPENDENT RESISTANCE TO VIRUS INFECTIONS

By their position at sites of initial infection (e.g., the respiratory tract) and their wide distribution in major organs of the body (e.g., the liver), macrophages may be decisive in determining the susceptibility or resistance of an animal to virus infections (98). The uptake or clearance of a virus particle from the circulation or extracellular fluids has been shown first of all to depend on the particle size (98), as is the case with inert particles (165), larger particles being cleared more rapidly than smaller ones. However, other factors, such as the presence of virus-specific receptor sites on macrophages and the presence of opsonizing antibody (25), are also important. By uptake and digestion of virus particles, macrophages may delay or even prevent the spread of the infection to susceptible cells. However, if the virus can replicate in macrophages, the infection may go further, and widespread destruction of organs and tissues may occur. Furthermore, infected monocytes in the circulation can, provided they support virus replication, be a source of dissemination of the infection by their migration through the body (45, 51, 98). It thus seems clear that virus-macrophage interactions may be of crucial importance for the outcome of the infection.

Much information on the importance of macrophages in resistance to virus infections has been gained by studying experimental infections in which differences exist between the interac-

tions of macrophages with different strains or types of the same virus. Likewise, models in which differences in handling a certain virus are found among macrophages from animals of different ages or different strains of the same species have been extensively studied. Aspects of these studies and the antiviral capacities of activated and stimulated macrophages are considered in the next sections.

Virus Strain- and Virus Type-Related Resistance

In studies of the pathogenesis of virus diseases, much valuable information can be obtained by comparing the pathogenic events in vivo and virus-cell interactions in vitro of closely related virus strains or virus types (10). In this way, it has been possible to recognize a number of virulence or pathogenicity markers of a particular virus and resistance determinants of the host (138). In some cases, the ability of a virus strain or virus type to replicate in macrophages from an animal has been shown to be correlated with virulence of that particular virus strain or type for the intact animal, whereas the ability of macrophages to restrict virus replication has been correlated with resistance of the animal. Both naturally occurring strain or type variants and virus strains obtained by adaptation to a particular host have been used. Of these, the naturally occurring strains or types seem to be most reliable in disclosing real resistance factors of the animal, since it cannot be ruled out that the various adaptation or attenuation procedures to which the laboratory strains have been subjected have resulted in mere artifacts with little bearing on the natural situation.

The earliest comparative study of the interaction of virulent and avirulent strains of a virus with macrophages in vitro was performed by Bang and Warwick (11). They examined the growth of a virulent strain and an avirulent vaccine strain of Newcastle disease virus in chicken cells. The virulent strain of the virus, which kills embryos and chickens rapidly, destroyed chicken fibroblasts and macrophages in culture, whereas the avirulent strain, which only causes mild respiratory infection in hatched chicks, grew poorly in chicken macrophages. Although the avirulent strain also grew less abundantly than the virulent strain in fibroblasts, the macrophage cultures showed a more consistent difference in susceptibility to the two strains of virus.

Roberts (121) used the differences in virulence for mice of two strains of ectromelia virus to analyze host defenses against liver infection by this virus. The Hampstead mouse strain, which had only been passed in mice, was found much more virulent than the Hampstead egg strain, which had gone through more than 60 egg passages. After intravenous inoculation, virus growth in livers was followed by infectivity titrations, and the number of infected Kupffer cells and the spread of the infection to parenchymal hepatic cells were estimated by fluorescence microscopy. It was found that the avirulent strain was much less efficient in infecting Kupffer cells and that Kupffer cells which were actually infected yielded less virus than did cells infected with the virulent strain. No difference was found in the ability of the two strains to grow and spread in liver parenchymal cells. These studies were followed up by quantitative in vitro studies of the susceptibility of mouse peritoneal macrophages to infection with the two virus strains. By the fluorescent antibody technique, it was found that peritoneal macrophages were about 10 times as susceptible to infection by the virulent strain of virus as they were to infection by the avirulent strain (122). No in vitro studies of the infectivity and growth of virulent and avirulent strains of ectromelia virus in other cell types were conducted to support the in vivo observation that the difference in infectivity was only expressed in macrophages.

A similar differential action of mouse Kupffer cells against two vaccinia virus strains was mentioned by Mims (97). The CL-R strain grew in Kupffer cells and thereby gained access to hepatic parenchymal cells. The CL strain, on the other hand, did not grow in Kupffer cells, which thereby protected hepatic cells from the virus. The intrinsic ability of the CL strain to grow in hepatic cells was, however, disclosed by inoculating the virus up the bile duct to infect parenchymal cells directly.

Tosolini and Mims (145) analyzed the behavior of two strains of lymphocytic choriomeningitis virus in mice. No antigenic differences have been demonstrated in different strains of this virus, and after intracerebral and footpad inoculation both the WE-3 and the Armstrong strains infected local tissues. However, after intravenous inoculation, only the WE-3 strain caused marked infection as detected by viral titrations and immunofluorescent and histological examinations of liver and spleen. Clearance studies revealed that the Armstrong strain was not detectably cleared from the blood after intravenous inoculation, whereas about 90% of the WE-3 strain was cleared within 5 min. Immunofluorescent examination of peritoneal macrophages harvested 2 days after intraperitoneal infection with the two strains of virus showed infection of 70% of macrophages obtained from mice injected with the WE-3 strain, whereas only 4% of the cells from mice inoculated with

the Armstrong strain were infected. After infection of macrophage cultures, the figures were 40 and 3%, respectively. It was concluded that the relatively inefficient uptake of the Armstrong strain by cells of the reticuloendothelial system was responsible for the inability of this strain to establish significant infection of the viscera, especially the liver.

MHV exists in several closely related serotypes with various degrees of pathogenicity in mice (88). In various studies, it has been found that a correlation exists between the pathogenicity of a certain type of the virus and its ability to grow in macrophages. Thus, the nonpathogenic type MHV-1 does not multiply in mouse macrophages (3), whereas the pathogenic types MHV-2 and MHV-3, which after intraperitoneal inoculation in mice produce fatal hepatitis, readily infect mouse peritoneal macrophages, in which they multiply to high titers with distinct cytopathic effects (13, 90). Likewise, a close correlation between the ability of two variants of MHV-2 to replicate in C3H macrophages and their virulence for C3H mice was described by Shif and Bang (136).

A specific pathogenic difference has been shown to exist between infections with HSV-1 and HSV-2 in mice. On intraperitoneal inoculation with HSV-2, progressive focal necrotizing hepatitis develops in most strains of mice, whereas HSV-1 only occasionally produces a few tiny, self-limiting foci of necrosis in the liver (Fig. 1) (106). This marker of pathogenicity, which has later been confirmed by others (120, 149), was shown to be very stable, as it was closely correlated with the antigenic type of the virus, irrespective of the anatomical site of primary virus isolation (genital or nongenital) or passage history of the strain. An in vitro correlate of the difference in pathogenicity between the two virus types was found in their abilities to replicate in peritoneal macrophages from mice (101). By an infectious center assay, it was found that although macrophages were rather restrictive in the replication of both virus types, the restriction of HSV-1 was far more pronounced than that of HSV-2 as judged by the number of plaques and progression of the infection in the target cell monolayer (Fig. 2). The difference in the yields of infectious virus from peritoneal macrophages infected with HSV-1 and HSV-2 was not caused by a relative inability of HSV-1 to adsorb to mouse macrophages, as the adsorption rates of the two virus types in macrophage cultures were equal. Moreover, it was shown that the more pronounced restriction of HSV-1 replication was specific for macrophages, as HSV-1 grew to higher titers than did HSV-2 in embryonic fibroblast cultures prepared from the same mouse strain. Further evidence in support of the participation of macrophages in the pathogenic distinction between liver infections with these two closely related virus types was obtained by selectively blocking the macrophage function of the animals by silica (104). Administration of 3 mg of silica intravenously 2 h before virus infection seemed to induce an effective blockade of liver macrophages

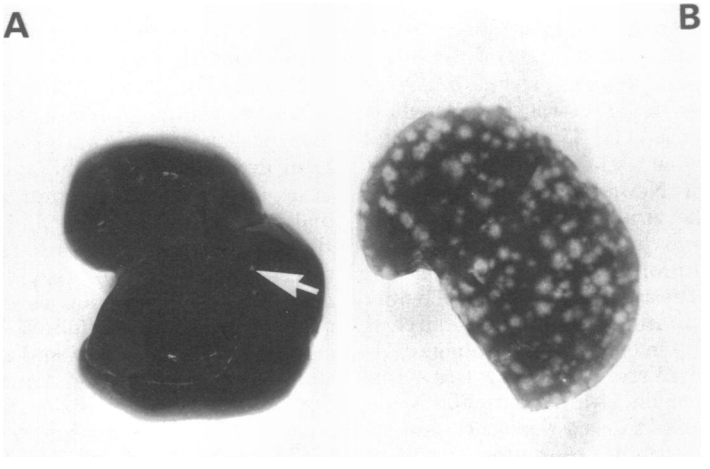

FIG. 1. *Livers from 3-week-old AH mice 4 days after intraperitoneal inoculation of* 2×10^4 *plaque-forming units of HSV-1 (A) or HSV-2 (B). A few tiny lesions are seen on the margin of the liver from the HSV-1-infected mouse (arrow). Magnification, ×2.5. From Mogensen (100).*

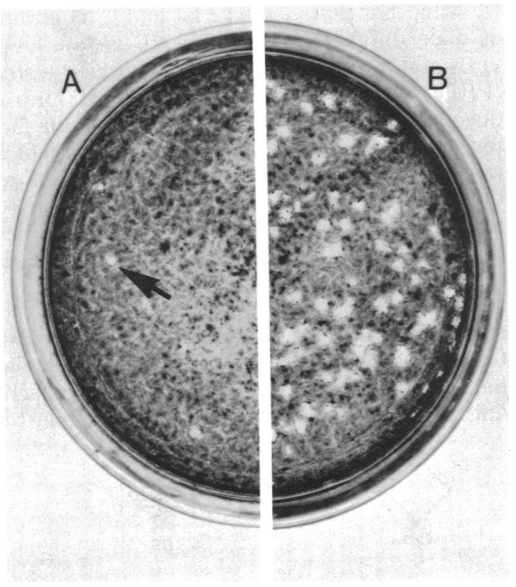

FIG. 2. *Infectious centers in macrophage cultures seeded with 5 × 10^5 peritoneal macrophages from 4-week-old BALB/c mice. The cultures were infected with 5 × 10^5 plaque-forming units of either HSV-1 (A) or HSV-2 (B), thoroughly washed, overlaid with susceptible mouse embryonic fibroblasts, and stained after 2 days of incubation. The arrow indicates a small HSV-1 plaque. Magnification, ×1.9. From Mogensen (101).*

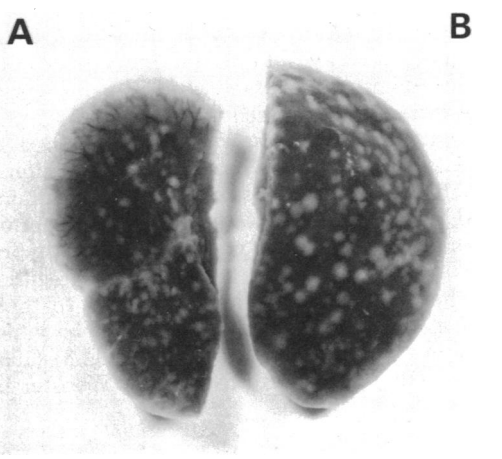

FIG. 3. *Livers from 4-week-old BALB/c mice injected intraperitoneally with 50 mg of silica 2 h before receiving 10^5 plaque-forming units of HSV-1 (A) or HSV-2 (B) intraperitoneally. The mice were sacrificed after 4 days. Magnification, ×2.5. From Mogensen and Andersen (104).*

for at least 2 days and a partial blockade for another 2 days, as HSV-1- and HSV-2-induced lesions progressed equally well for the first 2 days of infection followed by a relative retardation of the growth of HSV-1-induced lesions. Intraperitoneal inoculation of 50 mg of silica seemed even more effective in inducing macrophage blockade, as the difference in size and number of HSV-1 and HSV-2 lesions on day 4 of infection was almost abolished by this treatment schedule (Fig. 3).

Genetically Determined Resistance

It is a well-known fact that most viruses or groups of viruses differ in host range, some species of animals being susceptible to infection with a certain virus, whereas others are resistant. More interesting, however, is the variation in natural or innate resistance to infectious diseases displayed by members of the same animal species, indicating that factors in the genetic constitutions of the animals play an important part in the phenomenon (F. B. Bang, Adv. Virus Res., in press). Genetically determined resistance against infections is well documented as regards bacteriophages and plant viruses (1). The earliest reports on a genetic basis for resistance to animal virus infections were published more than 40 years ago (50, 87, 158). Since then, the inheritability of resistance to virus infections has been demonstrated in several animal model systems, and the genetics behind the variation has been explored. Instances in which resistance (48, 49, 82, 102, 127, 130) or susceptibility (13, 29, 112) is determined by a single dominant gene have generally offered the best prospects of elucidating the virus-host interactions involved, whereas polygenic traits (80, 85, 164) have been more difficult to evaluate.

Webster (158) was the first to work out the Mendelian ratios for the inheritance of resistance to a virus infection by crossing resistant and susceptible strains of mice followed by appropriate intercross and backcross matings. In Fig. 4 and 5, the expected ratios of susceptible and resistant animals are outlined in examples of virus infections where resistance or susceptibility is inherited as a monogenic autosomal dominant trait with genetic segregation of resistance and susceptibility occurring in the second filial (F_2) generation and in backcrosses (BC_1) to the recessive parental strain. When two or more loci are involved, genetic evaluation is more difficult because of the possibilities of interaction or linkage between the loci or varying contributions to the phenotype of the loci involved.

In some virus-animal systems, macrophages have been found to express at the cellular level the genetic resistance seen in vivo (Table 1). The best clarified examples of this are the re-

sistance of mice to flaviviruses (arbovirus group B) and the susceptibility of mice to MHV-2, both of which are inherited as monogenic autosomal dominant characters, thus representing the two cases illustrated in Fig. 4 and 5.

During the thirties, Webster developed by selective breeding strains of mice which differed greatly in resistance to two flaviviruses, St. Louis and louping ill viruses. By crossing susceptible with resistant lines of mice and testing hybrid and backcross progeny, he achieved segregation of the two characters in ratios close to those expected on the basis that resistance be inherited as a single, dominant factor (Fig. 4) (158). Sabin (127) extended the work of Webster by showing that nonselected PRI and C3H strains of mice differed in resistance to yellow fever virus and a number of other flaviviruses tested (PRI, resistant; C3H, susceptible). He introduced the terms "multiplication-depressing factor" and "cellular vulnerability," both of which were of importance for relative resistance. Furthermore, evidence was presented that inherited resistance operated at the cellular level, since virus multiplied freely in foreign tumor cells implanted in resistant PRI mice (128).

In extensive studies of the genetics of resistance to West Nile virus, another flavivirus, Goodman and Koprowski (48, 49) confirmed Sabin's findings and further investigated the mechanism of inherited resistance. After intraperito-

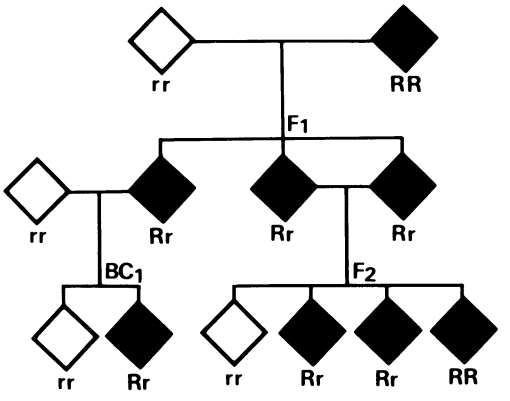

FIG. 4. *Autosomal dominant inheritance in mice of resistance to virus infection. The symbols indicate both female and male mice with the resistant (◆) or susceptible (◇) phenotype. RR, Rr, and rr indicate the genotypes. BC_1 is the first backcross generation in a test cross between mice of the F_1 generation and the recessive parental strain. In F_1 all mice are resistant, in F_2 75% are resistant, and in BC_1 50% are resistant.*

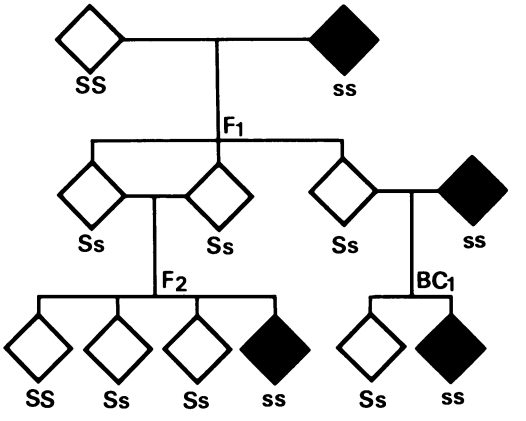

FIG. 5. *Autosomal dominant inheritance in mice of susceptibility to virus infection. The symbols indicate both female and male mice with the susceptible (◇) or resistant (◆) phenotype. SS, Ss, and ss indicate the genotypes. BC_1 is explained in the legend to Fig. 4. In F_1 all mice are susceptible, in F_2 75% are susceptible, and in BC_1 50% are susceptible.*

TABLE 1. *Inheritance of macrophage-dependent resistance to virus infection in mice*

Virus	Mouse strains		Property tested[a] (mode of inheritance)	Reference
	Resistant	Susceptible		
Flaviviruses	PRI, BRVR	C3H/He, BSVS	R (autosomal dominant)	48, 49
MHV-2	C3H/He	PRI	S (autosomal dominant)	13
MHV-3	A/J	C57BL/10, DBA/2, (C3H/He, A2G)[b]		155
HSV-2	GR/A	BALB/c	R (X-linked dominant)	102
Murine cytomegalovirus	CBA/J	C57BL/6	R (autosomal dominant)	132
Influenza A	A2G	A/J	R (autosomal dominant)	82

[a] R, Resistance; S, susceptibility.
[b] Intermediate susceptibility.

neal inoculation, the virus was cleared at comparable rates within 10 to 12 h from the blood of both susceptible C3H and resistant PRI and BRVR mice. In susceptible mice, however, a second period of viremia soon occurred, whereas virus did not reappear in the blood of resistant mice. Specific antibody production or putative nonspecific serum inhibitors could not account for this difference nor was any febrile reaction observed in the resistant mice. Attempts to modify resistance by various treatments (X rays, cortisone, Thorotrast, thioguanine, endotoxin) did not reveal the nature of the resistance mechanism. However, a cellular expression of inherited resistance was found in the capacity of cultures of splenic and peritoneal macrophages from resistant and susceptible mice to support virus multiplication. Whereas virtually no virus production was seen in cultures of these cells from resistant PRI and BRVR mice, titers of 10^2 to 10^4 plaque-forming units per ml were recovered from supernatants of cultures prepared from the susceptible C3H strain. Furthermore, resistance and susceptibility in vitro were found, as expected, to segregate close to 1:1 in macrophage cultures prepared from individual BC_8 mice (48). Gröschel and Koprowski (52) developed a homozygous inbred mouse line (C3HRV) which was congenic with the virus-susceptible C3H/He strain, only differing from the latter at the gene for flavivirus resistance. The new mouse line was found to be just as resistant as the PRI mouse from which the resistance gene had been transferred, and macrophage cultures reflected this resistance completely.

The basic nature of the depressed flavivirus multiplication in macrophages from resistant mice is not fully understood. Conflicting results exist as regards the specificity of macrophages as the only cell type expressing the genetics of resistance. In the original reports (48, 49), no difference was found in the capacities of kidney and lung cells from susceptible and resistant mouse strains to support virus multiplication, whereas later investigators (56, 150) claim that such a difference exists, although to a lesser and more variable degree than that displayed by macrophages. Differences in interferon production have been shown not to play any role in virus resistance: interferon levels were demonstrated to be lower in resistant than in susceptible mice (151), and interferon production levels in macrophage and fibroblast cultures from resistant and susceptible strains of mice were equal (56). However, cells from the virus-resistant mouse strain were more readily protected by interferon from virus infection than were cells originating from the susceptible strain (56). It thus appears that Sabin's inheritable multiplication-depressing factor may be the interferon sensitivity of the host cells, principally macrophages (constituting the primary barrier to successful virus invasion) but also the target cells for the infection.

MHV-2 infection in mice provides an example of susceptibility inherited as a dominant unifactorial trait (Fig. 5). In 1959, Bang and Warwick showed that this virus caused selective destruction of the macrophage population in outgrowths of liver explants from susceptible PRI mice, leaving both the parenchymal liver cells and the epithelial cells and fibroblasts intact (12). Interest in this phenomenon was greatly intensified when it turned out that C3H mice, which were resistant to the infection, yielded macrophages which were themselves resistant when infected in vitro (13). Tests of other strains of mice resistant or susceptible to MHV-2 in vivo showed complete agreement between mouse and macrophage susceptibilities. Genetic crosses between susceptible PRI and resistant C3H mice yielded F_1 offspring all of which were susceptible, indicating that susceptibility is the dominant feature. Segregation of the characters was achieved in the F_2 (3:1) and in the BC_1 (1:1) generations in ratios in accordance with a single gene for susceptibility. A similar segregation of susceptibility and resistance occurred in macrophage cultures prepared from individual mice of the F_2 and BC_1 generations (13). Further backcrosses showed that the PRI gene for macrophage susceptibility was constant and completely manifest even when present in a C3H (resistant) background: the plaquing efficiency of the virus in macrophage cultures from susceptible mice of the BC_7 generation was as high as that in PRI cultures (134); macrophage cultures continued to segregate in ratios close to 1:1 during 20 backcross matings; and macrophages obtained from the virus-susceptible inbred line of C3HSS mice, which are congenic with resistant C3H mice and only differ from these at the locus for MHV susceptibility, were just as susceptible as macrophages from the PRI mice, from which the gene for susceptibility was transferred (160). The expression of the genotype for susceptibility or resistance was not confined to liver macrophages, since the same picture emerged when macrophages were cultured from other tissues (13) or from peritoneal exudates (70, 71).

A further investigation of the differential abilities of macrophages from C3H and PRI strains of mice to support replication of MHV-2 was undertaken by Shif and Bang (135). They showed that the virus was adsorbed equally well

to resistant and susceptible cells. After adsorption, the virus disappeared into the eclipse phase in the susceptible macrophages and subsequently replicated to high titers within 20 h, whereas in the resistant cells the input virus persisted in an infective state for several days without multiplication and without causing any apparent damage to the cells. It thus appears that the resistance of C3H macrophages is related to the ability of these cells to maintain this particular virus in a nonreplicative state. In this connection it is of interest to note that the same two strains of mice have been used in studies of the genetics of macrophage resistance to both MHV-2 and flaviviruses but that the strain susceptible to MHV-2 is resistant to flaviviruses and vice versa. Furthermore, when C3H macrophage cultures are inoculated with high multiplicities of MHV-2, a variant virus emerges which is capable of killing C3H mice and of replicating in and destroying C3H macrophages (136). It is thus clear that the capacity of C3H macrophages to restrict the growth of the strain of MHV-2 used in these studies does not stem from a generalized capacity of C3H macrophages to restrict virus replication and that the importance of macrophage-virus interactions requires individual assessment with each virus-host system involved.

Several attempts have been made to elucidate the factor or factors which determine resistance and susceptibility of macrophage cultures to MHV-2. Evidence has been presented that it is a property of the individual cells: macrophage cultures from resistant C3H mice remained resistant after several changes of media, and infection of mixed cultures prepared from equal numbers of PRI and C3H macrophages resulted in the destruction of about 50% of the macrophages (13). Likewise, no sign of interferon activity was found in resistant C3H cultures after infection (136). However, a factor related to susceptibility was recognized in extracts from uninfected susceptible PRI macrophages. Resistant C3H macrophages exposed to this extract before infection with the virus became susceptible, whereas extracts from C3H macrophages did not change the susceptibility of PRI macrophages (70). Although the factor has been partly characterized (a relatively large, heat-stable molecule only slightly sensitive to deoxyribonuclease and insensitive to ribonuclease treatment), the nature of the conversion from resistance to susceptibility has not been revealed (71). Further attempts to modify the resistance of C3H mice have not thrown much light on the mechanisms by which macrophages display this character. Cortisone treatment rendered C3H mice partly susceptible to the infection, and macrophages treated in vitro with cortisone also became partly susceptible to the destructive effect of the virus (46), but a causal relationship between these findings was not established. Cyclophosphamide treatment likewise increased in vivo susceptibility, although the drug had no effect on in vitro replication of the virus in macrophages (162). It was shown that the conversion of resistance to susceptibility in vivo by the drug was not due to inhibition of specific antibody production, and the effect of cyclophosphamide remains obscure.

Recently, brief reports have pointed to a possible effect of T lymphocytes and lymphokines on the expression of the genetic resistance or susceptibility of macrophages to MHV-2: thus, supernatant fluid from allogeneic mixed lymphocyte cultures converted macrophages from resistant C3H mice to susceptibility to the virus (159), whereas supernatants of concanavalin A-stimulated lymphocyte cultures had the opposite effect, rendering macrophages from susceptible PRI mice resistant (161). Furthermore, concanavalin A-treated PRI mice showed increased resistance to the infection, and macrophages from concanavalin A-pretreated mice were resistant in vitro. The significance of these findings is at present unknown, but they clearly show that lymphocytes and products of stimulated lymphocytes can modify the inborn defense mechanisms.

Susceptibility of mouse macrophages to MHV-3 was also found to parallel the susceptibility of mice of different strains to infection with this virus (155). Most strains of mice were fully susceptible and developed fulminant hepatitis upon infection. When infected in vitro, macrophages from these mice showed extensive giant cell formation, and the virus replicated to high titers. Mice of the A strain, on the other hand, were completely resistant, and no growth of virus was detected in their macrophages, even by the sensitive immunofluorescence technique. C3H and A2G mice showed intermediate susceptibility both in vitro and in vivo with development of a persistent infection and late neurological involvement. No data on the mode of inheritance were reported, but it is interesting to note that the C3H mice, which are resistant to MHV-2, were found to be intermediately susceptible to MHV-3, again stressing the risk of drawing too hasty conclusions as to resistance mechanisms against even closely related viruses.

Resistance of mice to the development of focal necrotizing hepatitis by HSV-2 was found by Mogensen (100) to be under genetic control. A further analysis of the patterns of inheritance has revealed that resistance is determined by

one dominant gene (or a complex of closely linked genes) located on the X chromosome (102). This unique feature of sex linkage of resistance to a virus infection emerged from matings of resistant male GR mice to susceptible female BALB/c mice and backcross matings of resistant F_1 females to susceptible BALB/c males (Fig. 6). The characters for susceptibility and resistance segregated between male and female offspring of the F_1 generation (all males susceptible, all females resistant) and in segregated ratios of about 1:1 in mice of both sexes in the BC_1 generation (Fig. 7). A cellular expression of virus resistance was found in the ability of peritoneal macrophages from resistant mice to restrict the replication of the virus in vitro. The restriction was most pronounced in macrophages from GR mice, and this character was retrieved in macrophage cultures from female F_1 progeny. Moreover, a segregation of high and low restriction close to the expected 1:1 ratio was found in the BC_1 generation (Fig. 8). No difference was detected in the replication of the virus in embryonic fibroblasts from resistant and susceptible strains of mice.

As regards the genetics of resistance to other herpesviruses, Lopez (85) presented results which indicated that resistance to HSV-1 infection in mice is dominant but that it is governed by at least two, and probably more, pairs of genes. The marker for susceptibility/resistance

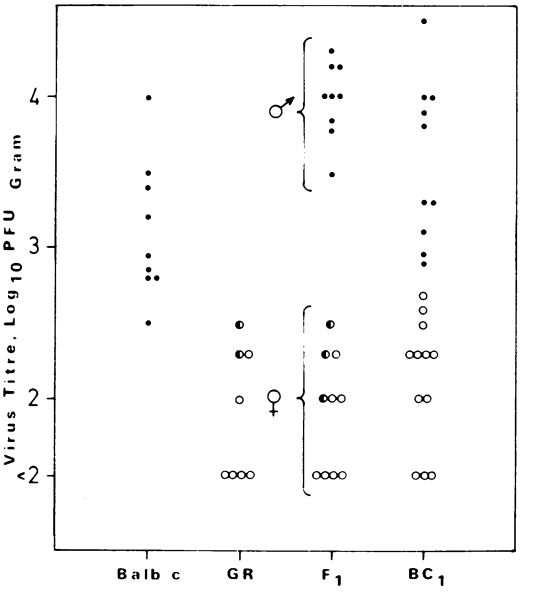

FIG. 7. *Titers of virus in livers of 8-week-old female BALB/c, GR, (GR♂ × BALB/c♀)F_1, and (BALB/c♂ × [GR × BALB/c]♀)BC_1 mice 4 days after intraperitoneal inoculation of 10^6 plaque-forming units (PFU) of HSV-2. Also shown are virus titers of F_1 male mice. Symbols: ●, mice with lesions; ○, mice without lesions; ◐, mice with tiny lesions on liver margins. From Mogensen (102).*

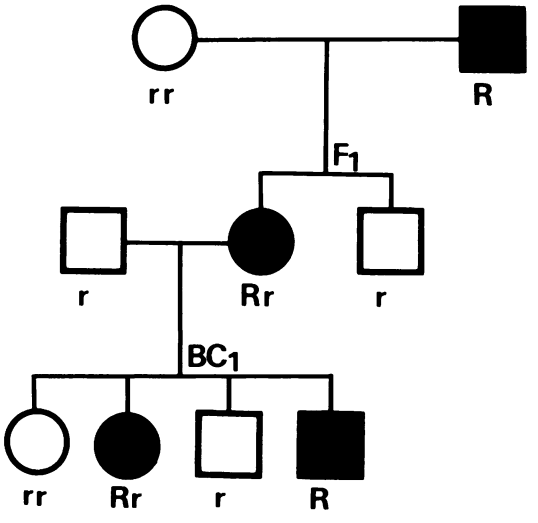

FIG. 6. *X-linked dominant inheritance in mice of resistance to virus infection in females (circles) and males (squares) with resistant (closed symbols) and susceptible (open symbols) phenotypes. For explanation of BC_1, see legend to Fig. 4. In F_1, all females are resistant and all males are susceptible. In BC_1, 50% are resistant irrespective of sex.*

was, however, the death of the animal, which is usually caused by central nervous system infection. This is a much more complex pathogenic event than infection of the liver and might imply hematogenic and neurogenic spread of virus to the brain. Indeed, several reports are available of polygenic inheritance of resistance to disease having a single-locus component at a certain point in the structure of resistance (63). Genetic control of resistance of mice to another herpesvirus, murine cytomegalovirus, was described by Selgrade and Osborn (132). They also found that resistance was the dominant feature, but it was independent of resistance to HSV-1 infection. Although transfer and blockade experiments suggested some importance of macrophages in host defenses against the virus, infection in vitro of macrophages from resistant CBA mice was no less productive than was infection of cells from susceptible C57BL mice. These findings do not support the concept of a simple barrier function of macrophages in genetic resistance to this infection, and it was proposed that the importance of macrophages in resistance was related to their role in the inductive phase of cellular immunity.

Resistance of A2G mice to various myxoviruses has been attributed to a single dominant

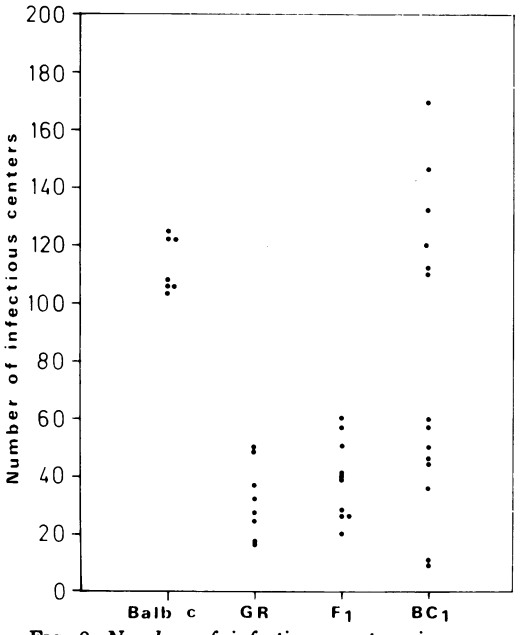

FIG. 8. *Number of infectious centers in macrophage cultures prepared from individual 8-week-old female BALB/c, GR, (GR♂ × BALB/c♀)F1, and (BALB/c♂ × [GR × BALB/c]♀)BC1 mice and infected with 5 × 10⁵ plaque-forming units of HSV-2. From Mogensen (102).*

gene designated *Mx* for myxovirus resistance (81, 83). The resistance is operative against neurotropic influenza viruses inoculated intracerebrally, pneumotropic strains injected intranasally, and a hepatotropic strain injected intraperitoneally (55). Attempts to define the phenotypic expression of the *Mx* gene have until recently been unsuccessful. No virus inhibitors were detected in body fluids or tissue extracts of resistant mice (83), and no difference has been found in the abilities of fibroblasts, kidney cells, and nerve cells from resistant and susceptible mice to support virus multiplication in vitro (82). Various immunosuppressive treatments failed to abolish resistance (55). However, recently, Lindenmann et al. (82) presented evidence that macrophages represent the key cell in the resistance of A2G mice to myxoviruses. An avian strain of influenza virus grew to high titers and caused a rapid and distinct cytopathic effect in peritoneal macrophage cultures from a number of mouse strains susceptible to influenza virus, whereas no signs of virus growth were detected in macrophage cultures prepared from resistant A2G mice or from F_1 hybrids between these and susceptible strains of mice. Furthermore, resistance and susceptibility of individual mice and their macrophages cosegregated in a ratio close to 1:1 in the BC_1 generation of resistant F_1 mice to a susceptible mouse strain. The fact that resistance is also manifest upon intracerebral challenge of A2G mice is not a common feature of infections in which macrophages have been found to be of importance in resistance; whether this phenomenon in the case of myxovirus resistance is due to such macrophage analogs as microglial cells in nervous tissue or to other mechanisms is at present unknown.

Even in cases where macrophages are of documented importance for resistance against virus infections, they do not represent the only defense mechanism. This was illustrated in the genetics of resistance of mice to ectromelia virus. Although virus growth in macrophages was found to be correlated with the difference in virulence for mice of two strains of this virus, the virulent virus strain grew equally well in macrophages from strains of mice with high, intermediate, and low resistance to infection (122). As pointed out by Schell (130), the differences in susceptibility of these strains of mice are probably attributable to differences in immune response, as differences in virus growth in the two strains of mice first appeared at a time when neutralizing antibodies and cell-mediated immunity were demonstrable. Furthermore, no difference was demonstrated in clearance of intravenously inoculated virus or in subsequent growth of virus in the liver.

Age-Related Resistance

A general characteristic of most virus infections in both humans (26) and animals (137) is the increased resistance to disease which develops in the course of growth and maturation of the host. The changes are particularly dramatic in the neonatal period. If the effects of antibody acquired passively from the mother are excluded, viral infections are often very severe in the neonatal period, becoming steadily milder during infancy and early childhood. In old age, infections again tend to become severe, presumably owing to progressing inadequacies of host defense mechanisms (89). Several explanations of the age-dependent increase in resistance have been advanced, including maturation of immunological reactivity, augmented interferon production, changes in viral receptors, and higher and more stable body temperature (44).

In many experimental viral infections, age-related resistance mechanisms are only operative after peripheral inoculation, both adult and neonatal animals being susceptible to intracerebral inoculation (113). This difference in susceptibility depending on the route of inoculation has led to the concept of development of "barriers" to the spread of virus from the periphery

to highly susceptible target organs (129). In an increasing number of experimental systems, evidence has accumulated which suggests that a maturation of macrophages occurs during the first few weeks of life. The mature macrophages of older animals seem to constitute a more effective barrier to the spread of virus than do immature macrophages from newborn and weanling animals. Examples of such age-related macrophage-dependent resistance to virus infections are given below.

In HSV-1 infections in mice, the development of age-related resistance to encephalitis after peripheral inoculation of the virus was first demonstrated by Andervont, in 1929 (7), and later confirmed by others (74, 79, 125). The existence of a cellular basis for the age-dependent resistance to this infection was claimed by Johnson in his comprehensive study of 1964 (69). By the use of the immunofluorescence technique, he showed that macrophages represent the cellular level at which the spread of extraneural infection is arrested in adult mice. Infection of peritoneal macrophages both in vivo and in vitro revealed that macrophages from adult and suckling mice were infected with equal ease. However, adult mouse macrophages infected in vitro did not spread the infection to other cells, whereas spreading of infection always occurred when suckling mouse macrophages were infected so that giant clumps of fluorescent cells had formed 72 h after inoculation. The in vitro capacity of macrophages to disseminate virus was shown to gradually decrease with the age of the macrophage donor in parallel with the development of resistance in vivo.

The importance of macrophage maturation in age-related resistance to HSV-1 infection was later confirmed by Allison's group. Selective blockade of macrophage function by silica and antimacrophage serum rendered weanling mice, which are relatively resistant to 10^4 plaque-forming units of HSV-1 much more susceptible to this virus dose, with a resulting wide dissemination of the virus and early death (167). Using an infectious center assay, Hirsch et al. (62) found that infected macrophages from suckling mice consistently released more virus than did adult mouse macrophages. Furthermore, intraperitoneal transfer of peritoneal macrophages from adult mice protected suckling mice from subsequent infection with a low dose of virus (10 plaque-forming units), but only when more than 6×10^6 stimulated macrophages were transferred. Smaller numbers of stimulated macrophages or large numbers of unstimulated macrophages did not alter the total mortality significantly, although a delay in the occurrence of death was seen. These findings might explain why Johnson (69) in his original investigations was unable to transfer augmented resistance by intraperitoneal transfer of macrophages. He used smaller numbers of unstimulated macrophages from younger donors and measured total mortality, which seems a rather insensitive parameter for macrophage protection (103).

Focal necrotizing hepatitis in mice caused by HSV-2 represents another model system in which the role of macrophages in age-related resistance has been investigated. On intraperitoneal inoculation with this virus, numerous focal areas of necrosis develop in the livers of young mice up to the age of 4 weeks (106). When older mice are infected, fewer animals show lesions, and the number and size of the foci diminish (100). Whereas the studies of age-related resistance to HSV-1 encephalitis have focused on the increase in resistance from the neonatal period to the age of 3 to 4 weeks, when the mice are weaned, the hepatitis model has revealed that mice do not reach maximum resistance to this infection until they reach the fertile age, i.e., about 8 weeks of age. In vitro infection of peritoneal macrophages from 3-week-old and 8-week-old mice showed that age-related resistance was concomitant with an increased restriction of replication of the virus in peritoneal macrophages, as judged by the number of plaques appearing in an infectious center assay. Furthermore, age-dependent resistance to HSV-2-induced hepatitis could be overcome by pretreatment with silica. Transfer of 2×10^6 unstimulated macrophages from 8-week-old mice to 3-week-old mice by the intravenous route conferred to the latter a defense capacity against HSV-2 hepatitis almost as effective as that seen in adult mice (103). One reason for this good rate of protection as compared with the results of Johnson (69) and Hirsch et al. (62) probably is that death from encephalitis, as used in their studies, is less sensitive than the degree of hepatitis as a marker for macrophage protection. When the virus enters the central nervous system, it seems to be beyond the reach of the host defense mechanisms which terminate the infection in peripheral sites, probably because macrophages are less effective in the central nervous system than in the organs of the mononuclear phagocyte system (2). Furthermore, Roser (123, 124) has shown that approximately 60% of peritoneal macrophages injected intravenously in mice are localized in the livers of the recipients within a few hours of injection, so that the intravenous route of transferring cells might be particularly suited to protect the liver from subsequent infection.

The human correlate to these animal model infections is neonatal infection with HSV (109).

The infection is usually acquired during the passage through the birth canal and hence is most often caused by HSV-2, which is the genital type of the virus. The infection is characterized by widespread dissemination of the virus with lesions in many organs, including the central nervous system and the liver, and is most commonly fatal. An unproportionate number of cases are seen in premature infants (1:1.4 against the expected 1:8 to 1:10), suggesting that immature host defenses (for example, macrophage function) are responsible for the ease of contracting a disseminated infection and developing overt disease. However, once established, the herpetic infection in full-term infants seems to be as severe as that observed in prematures (109).

Murine cytomegalovirus, another member of the *Herpesvirus* family, is similar to HSV in that suckling mice are much more susceptible to infection than are adults (91). The acute stages of infection are characterized by focal necrosis both in the liver and in other viscera. Henson et al. (58) showed that accumulation of Kupffer cells around lesions in the liver coincided with a decrease in virus titer and preceded the appearance of neutralizing antibodies by several days, suggesting that macrophages limit the spread of the virus in the livers of adult mice. This idea was supported by the finding of Selgrade and Osborn (132) that macrophage blockade with silica increased the susceptibility of adult mice to the infection. Furthermore, pretreatment of suckling mice with 10^6 stimulated adult macrophages significantly increased their resistance to subsequent murine cytomegalovirus infection. Pretreatment with 10^7 to 10^8 nonadherent splenocytes (lymphocytes) also conferred some protection to suckling mice. However, from their results it cannot be ruled out that this effect might be due to an even slight (1%) contamination of the lymphocyte suspension with macrophages. As regards the ability of the virus to replicate in murine macrophages, conflicting results have been reported: the data of Selgrade and Osborn showed only low levels of virus replication in murine macrophages, irrespective of the age of the macrophage donor; in contrast, Tegtmeyer and Craighead (142) achieved extracellular virus titers in macrophage cultures from adult mice just as high as those seen in cultures of mouse embryonic fibroblasts. However, the delayed appearance of virus in the macrophage cultures (6 to 12 days) as compared with fibroblast cultures ($\leq$ 3 days) renders it possible that outgrowth of fibroblasts in the macrophage cultures might be responsible for the high virus titers obtained in these experiments.

Resistance of mice to MHV-2 is genetically determined and closely correlated with resistance of macrophages in vitro (13). However, in the first few days of life, even genetically resistant C3H mice are susceptible. The ontogeny of macrophage resistance to this infection was studied by Gallily et al. (47). Although newborn C3H mice were susceptible to the infection, their survival time (6.5 to 8.0 days) exceeded that of "genuinely" susceptible PRI mice (2 to 4 days), and hence they were described as delayed susceptible. Resistance rapidly increased with age so that 14-day-old mice were fully resistant. The delayed susceptibility of infant C3H mice was reflected in the in vitro susceptibilities of liver and peritoneal macrophages explanted from mice of different ages. The maturation process occurred only in intact animals, as continued growth in vitro of liver macrophages from 1-day-old C3H mice did not increase the resistance of macrophages to the destructive effect of the virus, although the in vitro conditions of cultivation were able to modify the degree of resistance (47, 77). In susceptible C3HSS mice, which are congenic to resistant C3H mice, a temporary increase in resistance as the mice passed 7 weeks of age was noticed by Weiser et al. (160). The increased resistance in vivo was, however, not reflected in increased resistance of peritoneal macrophage cultures from mice of this age and was ascribed to incomplete penetrance of the dominant gene for susceptibility.

Most strains of mice are highly susceptible to infection with yellow fever virus by the intracerebral route, but not by the intraperitoneal route. Suckling mice, on the other hand, are highly susceptible by either route (143). Impairment of macrophage function by silica was found to increase the susceptibility of adult mice to peripherally inoculated virus (168). Similar results have been obtained by administration of antimacrophage serum (115). Passive transfer of specific antibodies to silica-pretreated mice 1 to 2 days after virus inoculation was fully able to substitute this function of macrophages, whereas administration of antilymphocytic serum to normal mice had no potentiating effect on the infection (168). These data suggest that macrophages are important in preventing the access of virus to the central nervous systems of adult mice, with antiviral antibody (normally first appearing on day 3 of infection) as a second line of defense. Cell-mediated immune reponses, on the other hand, seem not to be important.

The nature of the maturation process that renders macrophages from adult mice able to confine virus infections is not understood at present. The question has been most fully inves-

tigated in the case of HSV. A general cellular maturation cannot explain the limitation of virus spread, since cells of ectodermal, mesenchymal, and endodermal origin remain highly susceptible to productive infection (69, 141). No evidence of interferon production by virus-infected adult mouse macrophages was found by Johnson, and HSV-1-infected macrophage cultures were susceptible to rechallenge with the same virus or ectromelia virus (69). These findings were confirmed by Hirsch et al. (62); however, their data indicate that adult mouse macrophages, in contrast to suckling mouse macrophages, do produce low levels of interferon when infected with HSV-1, but that the interferon produced is ineffective in protecting other adult macrophages from being infected. The significance of these findings seems unclear, but probably interferon production is not the factor responsible for age-dependent maturation. Similarly, attempts to modify the capacity of macrophages to spread or contain infection by extracts of adult or suckling mouse macrophages analogous to the transfer of susceptibility to MHV among macrophage cultures from inbred strains of mice as described by Kantoch and Bang (70) were not successful (69).

The molecular and morphogenetic events in the restricted replication of HSV-1 in adult macrophages were systematically analyzed by Stevens and Cook (141). They concluded that the virus undergoes an abortive infection in these cells. Both viral DNA and the major virus-specified proteins were synthesized, yet electron micrographs of infected macrophages showed a paucity of virus particles with central dense cores and very few enveloped capsids. This suggests that virus-specified macromolecules are either structurally defective or produced in insufficient amounts or that errors exist in the assembly of the virus particle. Furthermore, it was shown that infected macrophages release virus-destructive products, probably lysosomal enzymes, which are abundant in these cells.

Recent studies have revealed differences in the ultrastructures of peritoneal macrophages of newborn and adult mice (57). The most conspicuous difference in the intracellular organizations of adult and newborn mouse macrophages is concerned with the rough endoplasmic reticulum, which is abundantly represented in adult mouse macrophages but is practically nonexistent in macrophages from newborn mice. Furthermore, differences were revealed in the organizations and charge densities of the cell membranes. Whether the differences reported represent significant elements of the different antimicrobial potencies of macrophages from newborn and adult mice remains, however, to be elucidated.

Role of Nonspecifically Stimulated and Activated Macrophages in Resistance

Besides constituting an immediate, "ready to function" barrier to virus penetration and dissemination in the body, macrophages participate in the defenses mounted by an infected host and directed against the invading pathogen. During the courses of many infections, macrophages become hyperactive and activated with increased microbicidal capacity. This process is immunologically mediated by specifically sensitized T lymphocytes, presumably via the secretion of soluble lymphokines, and macrophages are now recognized as important ultimate effectors of both antiviral and antibacterial cell-mediated immunity (23). The increased antimicrobial activity is at least partly nonspecific in that activated macrophages arising during an infection with one microorganism show increased activity against other, unrelated agents as well (20). In addition to increased microbicidal capacity, activated macrophages have been shown to exhibit a number of other distinctive features: increased phagocytic ability, accelerated "spreading" on glass surfaces (20), increased lysosomal enzyme content (31, 119), augmented metabolic functions (72), and antitumor activity (59). Many of these properties are shared by nonspecifically stimulated macrophages elicited in vivo or in vitro by various irritants, such as mineral oil, glycogen, proteose-peptone, thioglycolate, etc. (36), and it has been speculated that activated and stimulated macrophages are fundamentally alike (20).

It is well documented that specific macrophage recruitment and activation occurring in the course of a virus infection are of crucial importance for recovery (23). Much more open questions are the significance of nonspecific macrophage activation and stimulation in the natural resistance of the host to virus infections and the prospects of nonspecific macrophage-activating immunomodulation in the prophylaxis and treatment of virus infections.

In vitro, both stimulated and immunologically activated macrophages have shown great antiviral potentials. Thus, Hirsch et al. (62) demonstrated by an infectious center assay that proteose-peptone-stimulated peritoneal macrophages from adult mice were considerably more restrictive in the replication of HSV-1 than were unstimulated cells. Suckling mice, on the other hand, did not respond to proteose-peptone stimulation with the production of a macrophage population with these characteristics, a fact that

might partly explain the inefficiency of suckling mouse macrophages to restrict replication of viruses in vivo and in vitro. In another assay system, Lodmell et al. (84) demonstrated significant inhibition of plaque formation and virus yield in HSV-infected rabbit kidney cells incubated with rabbit peritoneal leukocytes (mainly macrophages) elicited with sodium caseinate. The kinetics of the inhibition suggested that the antiviral effect of the stimulated macrophages was exerted through nonspecific damage of both infected and noninfected cells, thus preventing cell-to-cell spreading of the virus. A similar antiviral effect toward HSV-2, vaccinia virus, and encephalomyocarditis virus was demonstrated by Morahan et al. (107) with both activated and stimulated mouse macrophages, whereas normal unstimulated macrophages were relatively inefficient in preventing virus growth. As a last example, mouse peritoneal macrophages immunologically activated by previous inoculations of *S. aureus* showed a considerably increased capacity to restrict replication of an influenza A virus, as revealed by the development of hemadsorption and the formation of S and V antigens (133).

In spite of the marked antiviral potency exhibited by stimulated and activated macrophages in vitro, most attempts to increase the resistance of experimental animals to virus infections by administration of macrophage-activating immunostimulants have yielded results of only marginal significance. Thus, the highly reduced capacity of influenza virus to replicate in vitro in macrophages from *S. aureus*-treated mice was only reflected in a moderate, although significant, increased survival time after influenza virus infection of similarly treated mice, whereas no significant reduction of total mortality was achieved (133). Adult rabbits inoculated with live *M. bovis* (BCG) were only partially protected from the development of encephalitis after subsequent corneal infection with HSV-2 (76). Likewise, BCG conveyed no substantial protection after vaginal HSV-2 infection of rabbits (76) or adult mice (9), although a combination of macrophage activation and passive administration of HSV-2 antiserum showed a marked effect in the latter study, probably because of the opsonizing effect of the antibodies. Among several putative macrophage stimulators examined by Starr et al. (140), only live BCG administered 6 days before viral challenge was able to reduce the mortality among suckling mice inoculated intraperitoneally with HSV-2, whereas levamisole, staphylococcal phage lysate, and inactivated typhoid and *Brucella* vaccines were without effect. It is noteworthy that BCG was the only agent containing live organisms and that at least 6 days were required to achieve the state of increased resistance, suggesting that immunological mechanisms are involved in the activation process. As a consequence of the 6-day period elapsing before protection was noted, it is questionable whether an already disseminated human neonatal HSV infection, which usually runs a rapid fatal course (109), might be influenced by BCG administration. Since, however, the incubation period of the infection is about 6 days, BCG might still prove effective if administered prophylactically to newborns delivered vaginally from mothers with active genital infection at the time of delivery or to babies with localized infections. It has been claimed that BCG administration is capable of controlling recurrent genital herpes infection in a clinical trial (6).

Further evidence of the potential importance of activated macrophages in host defenses against viral infections has emerged from two artificial experimental animal models, namely, mice with the graft-versus-host (GVH) reaction and congenitally athymic nude mice. A common feature shared by these experimental animals is a combination of severe immunosuppression with concurrent nonspecific hyperactivity of the mononuclear phagocyte system, making them suitable to expose the potentials of the macrophage-mediated resistance against invading viruses.

The development of a GVH reaction in adult F_1 hybrid mice by inoculation of immunologically competent lymphoid cells from one of the parent strains has been shown to induce a state of immunological incompetence against both bacterial and viral antigens affecting both the humoral (19, 22, 66) and the cell-mediated (21, 22) immune responses. On the other hand, the phagocytic activity of the mononuclear phagocyte system is profoundly increased as measured by the clearance of intravenously injected colloidal particles (64). The antibacterial potency of these active macrophages has also been found to be enhanced in that mice with the GVH reaction were more resistant to infection with *Listeria monocytogenes* than were normal mice (21). As regards resistance to viral infections, Blanden (22) found that clearance of intravenously inoculated virus was significantly enhanced in mice with the GVH reaction and that virus growth in the livers and spleens of these mice was consistently lower during the first 4 days of infection as compared with controls. The overall mortality was, however, higher in the group of mice with the GVH reaction. These findings strongly suggest that the hyperactivity

of macrophages caused by the GVH reaction improved the primary protection of these organs by being more efficient in the uptake and destruction of invading virus. The ultimate superiority of normal mice in controlling infection was not manifested until 6 to 8 days after infection, when the necrotic foci in the livers of normal mice became smaller and more densely populated with mononuclear cells. This feature was not seen in the liver lesions of mice with the GVH reaction, probably because of inefficiency of cell-mediated recruitment of mononuclear phagocytes.

Recently, several authors have revealed evidence suggesting that the nude mouse with congenital thymic aplasia represents another animal model in which nonspecific macrophage activation can be studied. It is widely agreed that nude mice, because of their immunodeficiency, are highly susceptible to several naturally occurring bacterial and viral infections (53). However, in recent reports, the mice have been shown to possess unexpected enhanced primary resistance to experimental infections with a number of pathogens, including *L. monocytogenes* (30, 39, 166), *Brucella abortus* (30), *Salmonella typhimurium* (43), and *Candida albicans* (33). Analyses of the courses of infection have pointed to an increased microbicidal capacity of mononuclear phagocytes as the cause of the augmented resistance of nude mice to these pathogens, a view that has been corroborated by in vitro studies of the interaction of the microorganisms and peritoneal macrophages in vitro (30, 166). Moreover, peritoneal macrophages from nude mice have been shown to exhibit nonspecific cytotoxicity to tumor cells in vitro (93), a finding that is consistent with the observed low incidence of spontaneous tumors in nude mice (32, 126).

In 1976, Mogensen briefly reported an unexpected high resistance of nude mice to induction of focal necrotic hepatitis by HSV-2 (100). A later study (105) confirmed that nude mice beyond the age of 4 to 5 weeks were considerably more resistant than congenic mice of the background strain and phenotypically normal heterozygous littermates, differing in genetic constitution from the nudes by the *nu* gene only, which codes for thymic aplasia and nakedness. Studies of the courses of infection showed that nude mice were able to restrain virus multiplication in the liver far better than were normal mice during the first 5 days of infection (Fig. 9). In vitro investigation of peritoneal macrophages revealed that macrophages from 6-week-old nude mice exhibited accelerated spreading after 1 h in culture, a well-accepted characteristic of activation. Assessment of the replication of

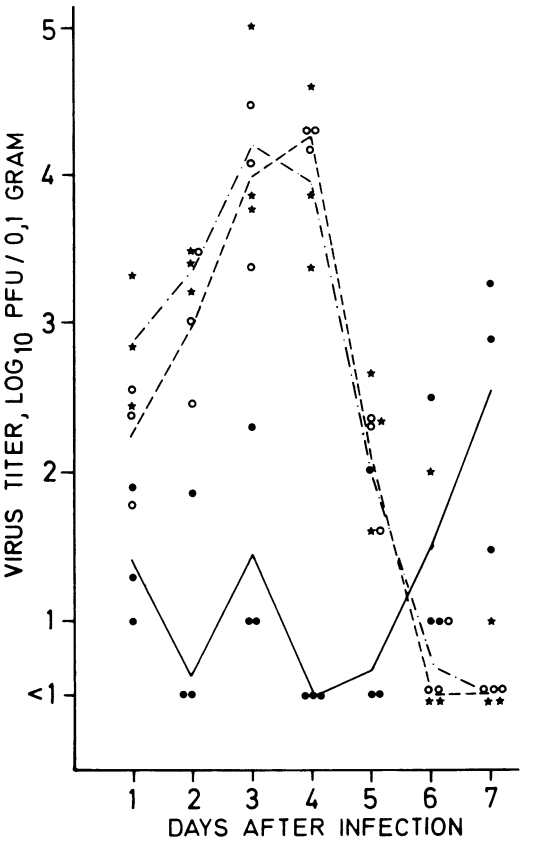

FIG. 9. *Courses of infection in the livers of 6-week-old homozygous nude (nu/nu) mice* (●, ——), *heterozygous (nu/+) littermates* (★, —·—·—), *and BALB/c mice* (○, — — —) *inoculated intraperitoneally with* 10^6 *plaque-forming units of HSV-2. Each point represents one mouse. Lines are drawn between the means of each group. From Mogensen and Andersen (105).*

HSV-2 in macrophage cultures by an infectious center assay revealed that macrophages from nude mice were much more restrictive in this respect than were macrophages from normal mice, since the number of macrophages from nude mice that supported virus replication was only one-third the number from normal mice. As further evidence of the role of activated macrophages in the resistance phenomenon, it was found that blockade of the mononuclear phagocyte system by silica treatment abolished the increased resistance of nude mice. The causal relationship between the athymic state and resistance was established by showing that restoration of the T-cell function of the nude mice by thymus cell grafting resulted in a normal, nonelevated defense capacity of the mice. However, in spite of the increased native resistance of nude mice, they seemed inferior to normal mice in the

final elimination of the infectious process (Fig. 9), probably because of the absence of a cell-mediated immune response. Likewise, Starr and Allison (139) found that nude mice were more susceptible than normal mice to infection with murine cytomegalovirus as measured by overall mortality; however, during the acute stages of infection a relative sparing of the liver of nude mice was noted as compared with the severe hepatitis seen in immunologically intact animals.

The causal relationship between the athymic state of nude mice and the restriction of virus replication in their macrophages was also demonstrated by Rao et al. (118). In their assay system, vaccinia virus showed a 30-fold increase in titer in macrophage cultures from normal mice, whereas macrophages from nude mice failed to support virus replication. However, macrophages harvested from nude mice having received a thymus transplant from heterozygous littermates 5 to 6 weeks earlier replicated the virus to control levels. Macrophages from nude mice raised under germfree conditions did the same. Similarly, Meltzer (93) demonstrated that macrophages from germfree nude mice were not tumoricidal, as were macrophages from conventional nudes. This shows that environmental stimuli are involved in the phenomenon. It has been shown that nude mice have poorly developed Peyer's patches and a low level of gut immunoglobulin A plasma cells (54). Probably, the intestinal bacterial flora of nudes is responsible for a sustained nonspecific macrophage activation through the absorption of bacterial lipopolysaccharide and phospholipid, which are known to elicit macrophage activation in mice (42, 43, 65). In favor of this hypothesis is also the finding of Nickol and Bonventre (110) that antibiotic elimination of the intestinal bacterial flora reduced the augmented resistance of nude mice to a challenge with *L. monocytogenes*.

CONCLUDING REMARKS

In this survey, aspects of the importance of the mononuclear phagocyte system as a barrier to the establishment and dissemination of virus infection in the body have been presented. From an anatomical point of view, cells of the mononuclear phagocyte system are well suited for this function. They are strategically placed at the portals of entry of many viruses and are widely distributed in close contact with the circulating blood in most organs of the body, thus encountering the virus early in the infection. From a functional point of view, the ability of macrophages to take up and destroy invading virus particles may be of the utmost importance in the race between the destructive growth of the virus and the induction of a specific immune response. If, on the other hand, the virus is able to multiply more or less freely in macrophages, the "viral burden" and the destruction may have proceeded beyond the limit of the capacities of the specific immune responses, when they eventually turn up.

No attempt has been made in this review to assess the role of other phagocytic cells, such as polymorphonuclear leukocytes, in natural resistance to virus infections. Polymorphonuclear leukocytes do not constitute a fixed barrier system in the body, and they are generally not a very conspicuous feature in the pathological lesions produced by viruses. Although they are capable of taking up virus particles, there is no evidence that they play a very prominent role, if any, in the antiviral defense mechanisms of the body (98, 104).

The collaboration of macrophages with other host defense mechanisms against virus infections is a subject of current interest. For example, macrophages have been shown to be of importance in the production by specifically sensitized lymphocytes of immune (type II) interferon (41). However, the role of macrophages seems more important in the immune response. Thus, macrophages are recognized as important ultimate effectors of antiviral cell-mediated immunity (23). Probably, macrophages also play a role in the inductive phase of the immune response by capturing the virus and by processing the viral antigens and presenting them to lymphocytes for immune recognition (148).

As previously stated, the basic nature of the capacity of macrophages to restrict the replication of viruses is at present unknown. A better understanding of this question, together with more insight into the collaboration of macrophages with other host defense mechanisms, might open new fields of specific immunomodulation. This could be of importance in the prophylaxis and treatment of virus infections in persons with increased susceptibility, for example, premature infants and persons with genetically determined or induced immune deficiencies.

SUMMARY

The scope of this survey was to analyze the role played by macrophages in natural, innate resistance to virus infections.

The term "macrophage" was introduced in 1892 by Metchnikoff to designate mononuclear phagocytes resident in various organs and tissues. On the basis of morphology, function, and kinetics of development, all highly phagocytic cells and their precursors are today grouped in

one cell lineage called the mononuclear phagocyte system. By their wide distribution in most organs of the body and by their ability to take up and destroy virus particles, macrophages are well suited to function as a barrier to the establishment and dissemination of virus infection.

Most of the information on the importance of macrophages in natural resistance to virus infections has been gained by studying experimental infections in laboratory animals, especially mice. Aspects of these studies have been dealt with in four sections: (i) studies in which macrophage restriction of virus growth has been shown to be of importance for the difference in the resistance of animals to closely related virus strains or virus types; (ii) studies in which variation in innate resistance to a virus infection displayed by members of the same animal species (genetically determined resistance) has been correlated with the ability of the virus to replicate in macrophages from the animals; (iii) studies in which an age-related increase in resistance to a virus infection has been found to be caused by a maturation of macrophages occurring during the first few weeks of life (the mature macrophages constitute a more effective barrier to the spread of virus than do immature macrophages from newborn and weanling animals); and (iv) studies in which restricted virus replication in nonspecifically activated macrophages accounts for augmented resistance to virus infections.

Although the basic nature of the capacity of macrophages to restrict the replication of viruses is at present unknown, an increasing amount of data from studies of the categories above listed points to a key role of macrophages as a first line of defense at the portals of entry of many viruses or in target organs of crucial importance for the outcome of infections.

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