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GENETIC ASPECTS OF MACROPHAGE INVOLVEMENT IN NATURAL RESISTANCE TO VIRUS INFECTIONS

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1. Summary

Macrophages are thought to constitute an important element in the body's natural defense against invasion and dissemination of viruses. Possible antiviral mechanisms of macrophages are defined and referred to as *intrinsic*, i.e. the ability of macrophages to serve as a non-permissive barrier between the virus and susceptible cells and *extrinsic*, i.e. the ability of macrophages to affect the virus or virus replication in surrounding cells. Most studies on the role of macrophages in natural resistance to virus infections have been performed in animal models. An interesting aspect of many viral infections in animals is the finding of a genetically determined variation in natural resistance. Because of the availability of numerous inbred and congenic strains most studies on genetically determined resistance have been performed in mice. The classical examples are resistance to flaviviruses and susceptibility to mouse hepatitis virus, both of which are inherited as dominant, monogenic traits. With these viruses macrophage intrinsic restriction of virus replication has been found to express at the cellular level the genetics of resistance/susceptibility seen in the intact animal. Other examples, where macrophages have been implicated in genetically determined resistance include herpes simplex virus and influenza virus. The involvement of macrophages in natural resistance to these viruses is discussed in relation to other putative resistance determinants like interferon production and sensitivity and natural killer cell activity.

Key words: genetic resistance – macrophages – herpesvirus – flavivirus – coronavirus – myxovirus

2. Introduction

Natural defense mechanisms may be of the utmost importance in determining the outcome of a virus infection. They are either part of the normal constitution of the uninfected individual or are induced very early during the course of the infection. Natural defenses therefore have the opportunity to control the establishment of a virus infection and to interfere with the early dissemination of the virus.

A multitude of elements are involved in natural resistance to viruses. In recent years special interest has focused on three factors, namely interferon [1, 2], macrophages [3–5] and natural killer cells [6]. Although they probably work in concert to control the early phases of an infection, their relative roles in different virus infections and in different organs of a particular infection are still far from being settled.

3. Virus–macrophage interactions

Macrophages are well suited to function as an important element in the defense against invasion

of viruses [3]. They are long-lived and strategically placed at the portal of entry of many infections (e.g. the alveolar macrophages of the lung) and are widely distributed in most organs and tissues of the body in close contact with the circulating blood (e.g. Kupffer cells of the liver). Thus, they are in a position to encounter virus early in the infection.

Two kinds of interaction between viruses and macrophages have been defined in vitro, termed intrinsic and extrinsic virus–macrophage interaction, respectively [7].

3.1. *Intrinsic virus–macrophage interaction*

This refers to the outcome of virus replication in the macrophage per se. Macrophages may possess various degrees of permissiveness for virus replication. In the non-permissive situation macrophages represent a dead end for propagation of the infection. In the permissive situation the virus is able to replicate productively in macrophages and progeny virus particles are released to surrounding permissive cells. Furthermore, infected monocytes in the circulation may, by virtue of their migration through the body, serve as a vehicle for dissemination of the infection [3].

A number of variables in the experimental procedure may influence the outcome of the intrinsic virus–macrophage interaction [4]. Among these, mention may be made of the source of macrophages [8, 9], the use of non-specific irritants to increase macrophage yields [10] and culture conditions like the serum type and concentration [11], the presence of antiviral antibody [12] and precultivation of the cells [2, 10]. Macrophages are generally more restrictive for virus replication than most other cell types [4]. By infectious center assays it has been shown that even though the majority of macrophages may be infected (as detected by immunofluorescence), only a minor fraction of the cells release infectious virus. The basic nature of this phenomenon is still an enigma, and it is currently of major interest to relate permissiveness to any known particular heterogeneity of macrophages such as morphology, stage of cell cycle, stage of differentiation, or surface antigen expression [5].

In some cases it has been possible to correlate interferon production by macrophages with restriction of virus replication in these cells. Hirsch et al. [13] found that virus-restrictive adult mouse macrophages, in contrast to more permissive suckling mouse macrophages, produced interferon when infected with herpes simplex virus type 1. We have recently found that early interferon production by macrophages from mice resistant to herpes simplex virus type 2 is higher than interferon production by macrophages from susceptible mice and shows the same mode of inheritance as in vivo resistance and in vitro restriction of virus replication in macrophage cultures ([14] and Mogensen, to be publ.). The non-permissiveness of human monocytes for herpes simplex virus type 1 replication at a high multiplicity of infection has also been linked to interferon production in the cultures [15]. With some other viruses interferon production by macrophages has not been found to reflect virus replication in the cells. Thus, Hanson et al. [16] found that mouse macrophages with genetically determined differential ability to replicate flaviviruses produced equal amounts of interferon in response to these viruses. However, in the flavivirus–macrophage system, restrictive macrophages from resistant animals were more sensitive to the protective action of interferon than were more permissive macrophages from susceptible mice. Such a differential sensitivity to interferon between permissive and non-permissive macrophages has also been described for myxoviruses [2] and mouse hepatitis virus type 3 [17]. In a recent study on macrophage intrinsic restriction of vesicular stomatitis virus and encephalomyocarditis virus it was suggested that low, undetectable levels of endogenous interferon are maintaining an antiviral state in macrophages, since anti-interferon treatment of normal mice before macrophage harvest rendered these cells permissive to a subsequent challenge with the viruses [18]. A similar observation has been made with myxoviruses [2].

3.2. *Extrinsic virus–macrophage interaction*

This refers to the influence of macrophages on extracellular virus and virus replication in surrounding cells. Morahan and Morse [7] have out-

lined the various ways in which macrophages might theoretically interfere with all the steps in the replication of a virus in a permissive cell. The vulnerable stage of the infection will depend on the specific virus–cell interaction and therefore requires individual assessment with each virus–cell system. Special interest has focused on the question of whether interferon production by macrophages or direct macrophage cytotoxicity towards infected cells are involved. In the herpes system, which has been studied in detail, these mechanisms do not seem to be important, and it has been suggested that the effect is related to cytostasis through disturbance of host cell macromolecular synthesis [19]. A similar conclusion was reached by Hayashi et al. [20]. Alternatively, Wildy has suggested that arginase produced by activated macrophages might be responsible for the extrinsic inhibition of herpes simplex and vaccinia virus multiplication by depleting the medium of arginine, which is essential for replication of the viruses [21].

Extrinsic antiviral activity is generally not a prominent feature of normal, unstimulated macrophages [7, 20, 22], although it has been reported to occur in some instances [23, 24]. Therefore, extrinsic restriction of virus replication can probably not be considered a "ready-to-function" barrier to the establishment of virus infections in the sense that has been described for intrinsic restriction. However, since macrophage activation is an early event in many virus infections [7], extrinsic activity of activated macrophages accumulating in established foci of infection might be important to diminish further spread of the infection.

It is currently not known whether intrinsic and extrinsic restriction of viruses by macrophages are related phenomena. The fact that a given macrophage population may show a high activity in one and not the other character may indicate that this is not the case. For instance, resident macrophages are generally more intrinsically resistant to herpes simplex virus replication than thioglycollate-induced macrophages, whereas only the latter show high extrinsic restriction [5, 10]. Likewise, Stohlman et al. [24] were unable to correlate extrinsic and intrinsic macrophage antiviral activity toward mouse hepatitis virus. Preliminary information from the study of Wildy et al. [21] on the role of macrophage arginase in extrinsic restriction of viruses indicates that self-starvation of arginine may not be the effector mechanism in intrinsic restriction. However, a recent study has shown that γ-interferon-activated macrophages express intrinsic restriction and that this is related to arginase production, since addition of increasing doses of arginine to the macrophage cultures abrogated the induced restriction [25]. Probably, the effector mechanisms in both systems, and thus their relationship, may depend on the particular virus–macrophage pair studied and on the experimental protocol used.

4. Macrophages and genetically determined resistance to viruses

Natural resistance to virus infections often differs greatly among members of the same animal species [26]. In some instances a single dominant gene has been found to make a major impact on resistance or susceptibility. The classical examples of this are resistance of mice to flaviviruses and susceptibility of mice to mouse hepatitis virus type 2, both of which are inherited as monogenic autosomal dominant characters. The following will give examples of virus infections, in which macrophages have been found to express at the cellular level the genetically determined resistance seen in vivo.

4.1. *Flaviviruses*

During the thirties, Webster [27] developed by selective breeding strains of mice, which differed greatly in resistance to flaviviruses. Genetic analyses indicated that resistance was dominant and unifactorial. In the fifties, Sabin [28] extended this work on non-selected PRI and C_3H strains of mice and found PRI mice resistant and C_3H mice susceptible to a number of flaviviruses. The work of Sabin also indicated that resistance operated on the level of individual cells.

In extensive studies in the sixties with West Nile virus, Goodmann and Koprowski [29] concluded that the cells representing the phenotypic

expression of the gene for flavivirus resistance belong to the reticuloendothelial system. Thus, cultures of peritoneal and splenic macrophages from resistant and susceptible mice were found to differ greatly in permissiveness for the virus, and this trait segregated as expected in macrophage cultures from backcross mice. Also, macrophages from congenic resistant C_3HRV mice, only differing from susceptible C_3H mice at the gene for flavivirus resistance, expressed the resistance phenotype in vitro.

The depressed flavivirus replication in cells from resistant mice was found in the original reports to involve macrophages selectively. Later studies have found that other cell types, for instance brain cells and fibroblasts, also express flavivirus resistance [3]. However, this does not rule out a role for the macrophage barrier in resistance, but only implies that the resistance gene also operates, if the barrier has been broken.

The mechanism of action of the flavivirus resistance gene is not fully understood. Neither differential interferon production nor interferon sensitivity seem to play any particular role. Production of defective interfering particles by resistant cells may be of importance, although this may not constitute the only or even major mechanism, by which virus replication is blocked in resistant cells [30].

4.2. *Coronaviruses*

Infection of mice with mouse hepatitis virus type 2 provides the best example of susceptibility inherited as a dominant, monogenic trait. Studies over 2 decades by Bang and coworkers (reviewed in [26]) on mouse resistance to infection with this virus have yielded evidence that macrophages represent a cellular barrier to virus access to the liver. Genetic analyses of several strains of mice have shown a complete agreement between mouse and macrophage susceptibility to the virus. It is interesting to notice that the same two strains of mice (PRI and C_3H) have been used in studies of the genetics of macrophage resistance to mouse hepatitis virus and flaviviruses and that the strain susceptible to mouse hepatitis virus is resistant to flaviviruses and vice versa. This indicates that virus–macrophage interactions require individual assessment with each virus–host system.

Infection with mouse hepatitis virus type 3, another member of the coronavirus family, also differs in various mouse strains [17]. Most strains have been found to undergo a lethal, systemic infection with the virus, whereas the A mouse is fully resistant. C_3H mice show an intermediate susceptibility in that they resist the acute phase of the infection, but develop a chronic progressive disease. In all cases there is a precise correlation between the ability of the virus to replicate productively in macrophages with formation of multinucleated giant cells and the in vivo course of the infection. Resistance to the neurotropic mouse hepatitis virus strain JHM was also found to be correlated to intrinsic restriction of virus replication in macrophages, whereas macrophages from resistant and susceptible mice showed the same degree of extrinsic antiviral activity [24].

Several attempts have been made to elucidate the mechanism of macrophage restriction of mouse hepatitis viruses [17, 26]. Resistance is clearly expressed at the level of individual cells and interferon production by macrophages does not seem to be important. Although various manipulations of macrophage cultures have been found to modify the virus–macrophage interaction, these studies have not clarified the situation. On the basis of studies with anti-interferon treatment, Virelizier [17] has suggested that endogenous interferon, produced in response to the virus infection in question and also to past exposure to other stimuli may be essential for the acquisition of the ability of macrophages to restrict virus replication, and that the efficiency of interferon to do this varies in different mouse strains.

4.3. *Herpesviruses*

Studies of inbred mouse strains have revealed a marked difference in resistance among various mouse strains to both herpes simplex virus type 1 and type 2. Lopez [31] found resistance to herpes simplex virus type 1-induced encephalitis to be dominant and governed by at least two independent, non H-2-linked genes, whereas we

found resistance to hepatitis induction by herpes simplex virus type 2 to be influenced by one major X-linked dominant gene [32]. With both types of the virus, macrophages from resistant strains of mice exert higher intrinsic restriction of virus replication than macrophages from susceptible strains, a difference which is not seen in fibroblast cultures. In the case of herpes simplex virus type 2, macrophage restriction of virus replication correlated with resistance in the F_1 generation. Furthermore, high and low restriction segregated close to a 1:1 ratio in macrophage cultures prepared from individual mice in the BC_1 generation, indicating that a single gene is at work [32]. With the type 1 virus, Lopez and Dudas [10] were not able to relate the genetics of macrophage restriction of virus replication to resistance. Although F_1 mice were found to be resistant to the infection, macrophages from these mice replicated the virus as well as did macrophages from the susceptible parent. One reason for the different results obtained with the two virus types might well be found in the experimental approaches used. The results with type 2 were obtained with normal peritoneal macrophages infected in vivo or the day after plating in culture, whereas the results with type 1 were derived from thioglycollate-induced or 4-day-cultured macrophages, which have lost much of the native restriction-capability against the virus.

Suggestions for alternative mechanisms behind the genetically determined resistance to herpes simplex virus in the mouse have included NK cells and interferon production. Lopez [31] described an association between resistance and natural killing of herpes simplex virus type 1-infected targets by spleen cells. The nature of the effector cell(s) in the mouse has not been determined, but studies in humans have pointed to precursors of the monocyte–granulocyte series [33]. Genetically determined resistance to herpes simplex virus type 1 has also been related to very early interferon production, which is in part governed by an X-linked gene [34]. We have also shown that infection of resistant mice with high doses of herpes simplex virus type 2 elicits an early interferon response, which shows the same pattern of inheritance (X-linked, dominant) as macrophage-related resistance, suggesting an association between interferon induction and macrophage restriction [14]. Since interferon is known to activate NK cells and macrophages, early interferon induction may well be a central feature in genetically determined resistance to herpes simplex virus. Whether the link turns out to be genetically determined interferon production by macrophages, or interferon-induced, genetically determined activation of monocytes/macrophages to virus restriction or non-specific cytotoxicity is not clear at the moment.

4.4. *Myxoviruses*

Mice of the A2G strain show a pronounced and selective resistance to various orthomyxoviruses (reviewed in [2]). Resistance has been attributed to a single dominant gene *Mx*. For many years the phenotypic expression of the *Mx* gene was an enigma. In 1978 it was shown that peritoneal macrophages and Kuppfer cells from A2G and F_1 mice, as opposed to macrophages from a number of susceptible mouse strains, resisted replication of influenza virus. Furthermore, resistance and susceptibility of individual mice and their macrophages consegregated in a ratio close to 1:1 in back-crosses between resistant F_1 mice and susceptible mice, indicating that resistance in vivo and macrophage restriction of virus replication were related. However, in elegant studies with radiation chimeras it was later found that lethally irradiated Mx^+ mice substituted with macrophage precursors from susceptible Mx^- mice and vice versa expressed the resistance phenotype of the recipient, in spite of the fact that their macrophages expressed the phenotype of the donor. Thus, macrophage resistance and resistance of the intact animal did not seem to be causally related. Instead, evidence has been presented that the *Mx* gene influences the sensitivity of individual cells, including macrophages but also parenchymal cells, to an influenza virus-specific antiviral action of interferon.

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