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Contents

Interferon and Natural Killer Cells

Eero Saksela

Genetic Control of the Interferon System

Doris L. Slate, Frank H. Ruddle and Y. H. Tan

Mechanisms of Interferon Action: The (2′–5′)(A)$_n$ Synthetase–RNase L Pathway

Peter Lengyel

The Cloning of Interferon and Other Mistakes

Charles Weissmann

Present Appraisal of and Future Hopes for the Clinical Utilization of Human Interferons

Thomas C. Merigan

The Role of Interferon in Natural Resistance

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I IN SEARCH OF HEALTH

Medicine purports to maintain or restore health. An uncommitted observer from some distant planet might therefore expect biomedical research to be mainly directed at investigating health. Strangely enough this is not so: biomedical research is primarily concerned with disease.

It is true that physiology aims at elucidating normal function but it does so under highly abnormal conditions. Health is not simply an accretion of normal functions but the continuing effort to stabilize a permanently threatened equilibrium. Of course examples of such dynamic equilibria abound and physiology itself presents us with many. Thus, the seemingly static posture of a man standing upright can be dissected into its elements of motion and countermotion. In this case analysis is easy because the main force which impinges on the equilibrium, gravity, is known and constant.

When we come to the dynamic equilibrium we call health, we are quite ignorant of the actual stresses with which it has to cope. Some can be guessed, such as loss or accumulation of heat. Others may be surmised, like the ageing and functional degradation of certain cells or molecules. Still other possible disturbances are entirely hypothetical: do we have to imagine

our skin and mucous membranes as the site of a never-ending struggle between defence mechanisms and malevolant intruders, our inner organs as battle-grounds where malignant cells are ever so often summarily despatched?

With respect to any given disturbance, an equilibrium has a certain range within which regulation occurs and beyond which it breaks down. When disease is being studied, interest is focused on events past the breakdown point. In the field of experimental infection, conditions are usually so arranged that the result of intervention is reduced to a body count. Opposing forces at play before the breaking point is reached remain hidden. To reveal these forces, disturbances that stay well within the regulatory range have to be applied. However, in a well-tuned system like health the motions that re-establish balance are so swift and of such small amplitude that they can hardly be measured: rocking the equilibrium within its homoeostatic range does not result in any measurable reaction and it is therefore impossible to say whether the system is being rocked at all.

To give a simple example, if we apply a suspension of typhoid bacteria to healthy skin nothing happens. We may interpret this as showing that a tremendous challenge to our health, the encounter with fully virulent disease germs, has been successfully overcome by timely mobilization of protective forces. But we do not know if painting the skin with typhoid is in fact a challenge. To illustrate this point further: a car with pulled handbrake will not move when given either a longitudinal or a transverse push of a certain strength. But only the longitudinal push tests the quality of the breaks. In ignorance of the inner mechanics of the car, we would probably draw wrong conclusions from the observation that pushes in different directions yield the same result. Suppose now that certain types of cars were not fitted with handbrakes. These would show differences in stability depending on the direction of the push. From this we would learn that a longitudinal push truly challenges whatever opposes lengthwise movement in a parked car, whereas a transverse push would test a different mechanism.

Knowing now how to test longitudinal stability, we might try to damage certain systems in order to evaluate their role. The example I have chosen already gives an idea of the complexity to be expected: in the car fitted with a properly pulled handbrake, damaging clutch, gearbox, pistons or valves would hardly affect stability, but each of these manoeuvres would further destabilize the car without a handbrake. To see the function of the handbrake in a realistic perspective, as of major importance in preventing a parked car from rolling downhill but otherwise dispensable, would require a good deal of probing.

II GENETIC VARIANTS IN HOMOEOSTASIS

If we want to translate these simplistic considerations to the study of defence mechanisms operating in infectious diseases, we need the equivalent of cars of similar make, but with or without handbrakes, to know which challenges to apply. Most experimental infections have been selected to produce severe disease in laboratory animals; with respect to these infections laboratory animals behave like cars without handbrakes, and we have to look for the occasional animal that withstands a usually lethal infection. The reverse approach should also be successful, and there are examples from human pathology where the majority of people resist an infection and exceptional genotypes succumb to it. It is really an artefact of the experimental situation that leads to the impression that genetically determined susceptibility to an infectious disease is the rule and resistance is rare.

In the field of virology, the classical example of genetically determined resistance is that first observed 50 years ago by Sawyer and Lloyd (1931) and later extensively studied by the groups of Webster, Sabin and Koprowski (Bang, 1979). In this system various flaviviruses (formerly called arbor B viruses) will, when introduced by some appropriate route, kill the vast majority of inbred mice, but will spare members of some strains. That an adequate challenge is being applied is shown by the lethal effect on susceptible mice: the push is in the right direction to move the car. As to the mechanism which allows resistant mice to survive, isolating the equivalent of a handbrake has proved difficult, as can be surmised from the various explanations put forward by one and the same group over the years: thus, the immune system was implicated (Theis *et al.*, 1959); interferon was tentatively ruled out (Vainio *et al.*, 1961); cellular antibodies were suggested (Vainio and Koprowski, 1962); macrophages were thought important (Goodman and Koprowski, 1962); interferon temporarily gained favour (Hanson *et al.*, 1969); thermoregulation was implicated (Lagerspetz *et al.*, 1973) and dismissed (Darnell *et al.*, 1974); interferon was again pushed into the background with interfering viral particles now the primary suspects (Darnell and Koprowski, 1974; Brinton, 1981).

Hence a comparison between flavivirus-susceptible and flavivirus-resistant mice is not between cars with or without handbrakes, but more likely a comparison between a car with worn brake, clutch, gearbox and engine, and one where all these parts are in better shape.

III INBORN RESISTANCE TO ORTHOMYXOVIRUSES

A chance observation made in 1961 led to a model which may be unique in

revealing the role of interferon. Mice of the inbred strain A2G proved resistant to intracerebral challenge with otherwise lethal amounts of neurotropic influenza A virus (Lindenmann, 1962). These mice have a strange history: they were thought to be descended from strain A, but fresh genetic material must have been introduced between 1942 and 1950 by an illegitimate mating. A2G indeed shares approximatively half of the alleles studied so far with A, and in particular the major histocompatibility configuration H-2^a (Lindenmann and Klein, 1967). Resistance is governed by a single, dominant gene: *Mx* (Lindenmann, 1964). Whereas homozygous *Mx/Mx* animals develop resistance within a few days of birth, heterozygous *Mx/+* mice need longer to express resistance, depending somewhat on the challenge virus used. This shows how relative a term like "dominance" is: tested at, say, 5 days of age, resistance would appear to be recessive; at 5 weeks, dominant.

The difference between resistant and susceptible animals was not only seen upon intracerebral challenge with neurotropic virus, but also upon intranasal challenge with pneumotropic strains or intraperitoneal challenge with a hepatotropic strain. It was therefore felt that whatever caused resistance must be systemic and could not reside in some anatomical peculiarity of a single organ. The immune system, an obvious candidate, could be ruled out (Haller and Lindenmann, 1974; Fiske and Klein, 1975; Haller *et al.*, 1976). Macrophages expressed resistance *in vitro* when freshly prepared from the peritoneal cavity (Lindenmann *et al.*, 1978). However, upon prolonged cultivation all macrophages became uniformly susceptible. Macrophages taken from the peritoneal cavities of mice pretreated with anti-interferon serum were susceptible from the start (Haller *et al.*, 1979a). Whereas a causative role of macrophages in resistance became unlikely when bone marrow chimaeras between histocompatible resistant and susceptible mice proved to express the phenotype of the recipient and not that of the donor (Haller *et al.*, 1979b), the role of interferon had to be taken seriously. The arguments in favour of interferon as the crucial element in this form of resistance are the following:

1. Resistant animals treated with anti-interferon serum became fully susceptible (Haller *et al.*, 1979a).
2. Macrophages from resistant mice, which had become susceptible upon prolonged cultivation, could be rendered resistant to orthomyxoviruses by treatment with small doses of interferon. Macrophages from susceptible mice similarly treated remained unprotected (Haller *et al.*, 1980).
3. The same observation was also made with hepatocytes and embryonic fibroblasts from resistant and susceptible mice (Arnheiter *et al.*, 1980).
4. The differential effect of interferon on cells with or without *Mx* was only seen with orthomyxoviruses, but not with unrelated viruses such as EMC, VSV or herpes.

5. Newborn mice of genotype *Mx/+*, phenotypically susceptible during the first days of life, could be protected against lethal influenza virus challenge by small doses of interferon. Genotypically susceptible (+/+) newborn mice could not be similarly protected (Haller *et al.*, 1981a).
6. Disease produced in adult resistant animals by exceptional orthomyxovirus strains could be prevented by doses of interferon which were without effect on similarly infected susceptible mice (Haller *et al.*, 1981b).

This last point bears some elaboration. When the resistance spectrum of A2G mice was first established, we noted that resistance was manifested towards several influenza A and B strains and towards Sendai virus (Lindenmann *et al.*, 1963). Whether resistance against Sendai virus, a paramyxovirus, is dependent on the same gene has not been definitively settled; some degree of resistance to Sendai virus seems widespread (Stewart and Tucker, 1978). For the time being only resistance to strains of orthomyxoviruses can be firmly attributed to the gene *Mx*. However, even within the orthomyxo family some strains show a very large difference in pathogenic potential depending on presence or absence of *Mx*, whereas other strains show this to a lesser extent. A few strains, in fact, grow almost as well in *Mx/Mx* and +/+ mice and induce comparable pathology at the same virus dilutions. The first two strains seen to behave in this manner were both derived from A/Singapore/1/57 (Lindenmann *et al.*, 1963). These strains have been lost, and a later attempt at producing a mouse-adapted variant from a lyophilized ampoule of an early egg passage of the same strain yielded virus to which *Mx*-bearers were quite resistant. We therefore searched for other mouse-adapted strains labelled A/Singapore/1/57 and indeed found one which gave us the satisfaction of killing *Mx/Mx* mice as readily as +/+ mice. Detailed analysis of this strain revealed it as a close relative of A/PR/8/34, an old laboratory acquaintance. Other PR8 strains do show *Mx*-dependence, although sometimes not impressively. For instance, one PR8 virus tested originally reached almost the same endpoint titre on A2G and control mice, but at intermediary dilutions a prozone was observed in A2G mice only (Lindenmann *et al.*, 1963).

The availability of virus strains dependent or independent of *Mx* opens a new approach to the problem of resistance. Do these strains escape an interferon-induced antiviral state, or do they fail to induce interferon? The answer seems to be that they fail to induce interferon, for exogenously applied interferon does protect adult *Mx/Mx* mice; but a direct proof of defective interferon induction by these strains is still lacking. The prozone mentioned above could have been caused by a mixture of interferon-inducing and non-interferon-inducing virus.

IV THE COOPERATION BETWEEN *Mx* AND INTERFERON

How then does one account for resistance due to *Mx*? Cells possessing this allele are intrinsically as permissive for orthomyxoviruses as are cells devoid of it. Virus replication induces the formation of comparable amounts of interferon in both types of cells. This interferon in turn leads to antiviral states which are similar in both types of cells as far as viruses outside the orthomyxo group are concerned, but differ greatly when measured by challenge with influenza virus: in that case only cells equipped with *Mx* are extensively protected, whereas other cells remain susceptible. Macrophages from conventionally reared mice seem to enjoy a certain baseline level of "interferonization", which protects *Mx*-bearing macrophages even before their first encounter with virus. But this alone is not sufficient to protect the whole animal. Newborn mice are susceptible because they fail to produce sufficient amounts of interferon; given exogenous interferon, they are protectable according to their *Mx* status. Certain strains of virus apparently do not induce interferon and are therefore virulent for *Mx*-bearing mice; they can be held in check by exogenous interferon or interferon inducers.

Many interesting questions remain. How does spontaneous interferonization of macrophages come about and does it vary with age, diet, intestinal flora, subclinical infections, viral latency, carriage of endogenous viruses? How do certain virus strains avoid inducing interferon? And, perhaps the most interesting question: how does the *Mx* gene product collaborate with interferon to bring about an antiviral state which is spectacularly active against orthomyxoviruses?

On this last point I wish to indulge in some speculations. The *Mx* gene product is either constantly being made or it is switched on by interferon. If constantly present it could be an antiviral factor in its own right, blocking a metabolic pathway by which orthomyxovirus synthesis bypasses the interferon-induced antiviral state. Thus in cells lacking *Mx* interferon would be an inefficient inhibitor because orthomyxovirus synthesis would continue through the bypass channel, but in cells containing *Mx* the bypass channel would be permanently blocked, and interferon would be an efficient inhibitor. This is schematically illustrated in Fig. 1.

Another possibility would be for *Mx* to code for an interferon receptor. Suppose that different interferons induce different antiviral states which impinge more or less on different virus replication channels. IFN-x, for instance, would induce an antiviral state, blocking the orthomyxo replication channel, but to do so would require a receptor coded for by *Mx* (Fig. 2). This would require different receptors for different interferons, something which seems rather unlikely (Aguet and Blanchard, in press).

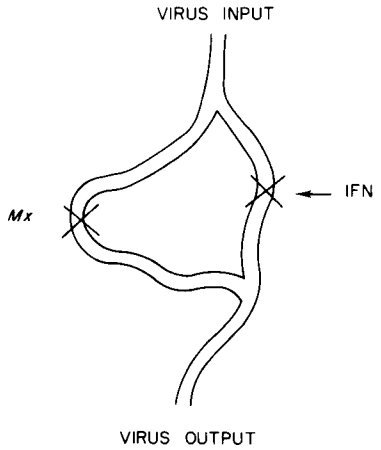

Fig. 1 Bypass hypothesis. In ordinary (+/+) mice interferon blockage of the usual replication channel for orthomyxoviruses (right channel) is bypassed (left channel). In *Mx*-bearing mice this bypass channel is permanently closed, and interferon is therefore a more efficient inhibitor of virus replication.

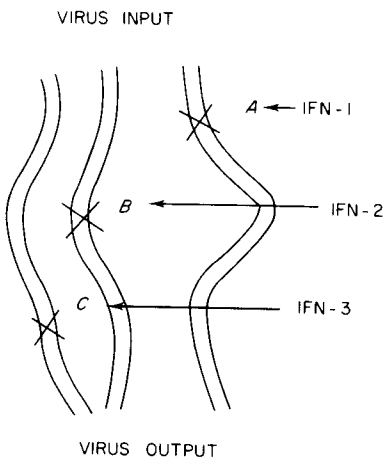

Fig. 2 Multiple interferon receptor hypothesis. The three paths represent independent replication channels for three groups of viruses. Genes *A, B, C* . . . each code for different receptors for interferons 1, 2, 3 . . . Each interferon induces an antiviral state which is specific for one replication channel only.

Lastly, the *Mx* gene product could be switched on only when interferon is present, and contribute to the antiviral state in such a manner as to render it efficient in blocking orthomyxoviruses (Fig. 3).

These three hypotheses, and several others that could be formulated, require that there be something unique in the replication of orthomyxoviruses—a channel distinct, at least at one point, from other replication channels through which other groups of viruses proceed. Advances in the clarification of the molecular biology of viruses should soon permit educated guesses as to what constitutes the sensitive event which is the target for the joint action of interferon and *Mx*.

That the ranking of viruses according to their interferon sensitivity depends on the test system used has long been recognized. Thus, vaccinia was the most interferon sensitive of five viruses in mouse and hamster cells, but the least interferon sensitive in rabbit cells (Stewart *et al.*, 1969). In these experiments both the cell type and the interferon preparation varied, which makes it difficult to decide which factor determined ranking. However, even within the same cell type, interferon sensitivity of different viruses varied with the degree of differentiation (Nilsen *et al.*, 1980). The antiviral state does not decay at the same speed for different viruses, and ranking may therefore depend on whether early yields or multiple viral cycles are being measured (Hallum *et al.*, 1970). Cell clones derived from the same origin have been described in which two viruses were similarly inhibited by interferon in one clone, and very dissimilarly in the other

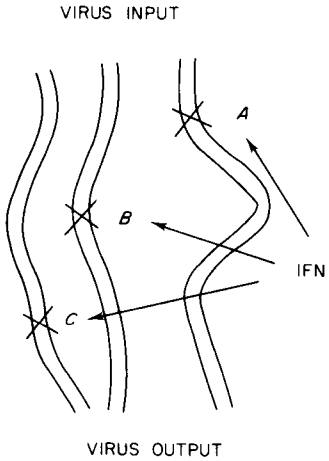

Fig. 3 Modulation of antiviral state hypothesis. The antiviral state is brought about by activation of genes *A, B, C* . . . each having its main effect on one replication channel only. Each channel allows replication of one group of viruses.

(Czarniecki *et al.*, 1981). Here the ranking is definitely dependent on the host cell.

In the *Mx* system very clear cut differences could be shown. Whereas in cells devoid of *Mx* influenza viruses can hardly be inhibited by interferon, in *Mx*-bearing cells they belong to the most interferon sensitive viruses. Depending on which cell is used very divergent opinions could be formed with respect to the interferon sensitivity of influenza virus. The same holds true for experiments *in vivo*: tested in *Mx*-bearing mice interferon would seem an efficient anti-influenza agent, and a very poor one in non-*Mx*-bearing mice. Such findings have obvious relevance for possible human applications of interferon.

V GENERAL IMPLICATIONS OF THE *Mx* SYSTEMS

Mx-governed resistance presents us with an example where interferon is the essential mediator. How far can such an insight be generalized? Obviously not by expecting that all instances of natural resistance will ultimately be reduced to the same mechanism. If interferon appears to be the dominating factor in resistance of mice to orthomyxoviruses, it is only an auxiliary factor in resistance to flaviviruses or in resistance to mouse hepatitis virus. That it plays a mitigating role within the more limited homoeostatic range of other virulent infections is seen from the effect of anti-interferon serum: infections with Semliki Forest, encephalomyocarditis, herpes simplex, Moloney sarcoma, vesicular stomatitis, Newcastle disease, polyoma, mouse hepatitis viruses all run a more severe course under anti-interferon globulin (Fauconnier, 1970; Gresser *et al.*, 1976a, b, 1979; Virelizier and Gresser, 1978). Interestingly, anti-interferon globulin was found not to alter influenza virus infection. Ordinary mice (i.e. lacking *Mx*) were employed in this study, suggesting that the mouse insensitive to interferon-mediated inhibition of influenza virus represents an exceptional state of affairs, and that the *Mx* carrier is more "natural".

That interferon cannot be held responsible for resistance in every case is most dramatically illustrated by those instances in which it contributes to pathogenesis: infection by lymphocytic choriomeningitis virus is rendered less severe by anti-interferon serum treatment (Rivière *et al.*, 1977; Gresser *et al.*, 1978). No sweeping statements are possible in this area, and each example deserves painstaking analysis. How complicated the situation really is becomes clear when the following facts are kept in mind: virus strains vary in their ability to induce interferon, and animals vary in their ability to respond to interferon-inducing stimuli (De Maeyer and De Maeyer-Guignard, 1979); virus strains also vary with respect to their interferon sensitivity, and this itself depends, as we have just seen, on the