SCIENCE

Immunological Tolerance

The phenomenon of tolerance provides a testing
ground for theories of the immune response.

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"Immunological tolerance" may be described as a state of indifference or nonreactivity towards a substance that would normally be expected to excite an immunological response. The term first came to be used in the context of tissue transplantation immunity—that is, of the form of immunity that usually prohibits the grafting of tissues between individuals of different genetic make-up —and it was used to refer only to a nonreactivity caused by exposing animals to antigenic stimuli before they were old enough to undertake an immunological response. For example, if living cells from a mouse of strain CBA are injected into an adult mouse of strain A, the CBA cells will be destroyed by an immunological process, and the A-line mouse that receives them will destroy any later graft of the same origin with the speed to be expected of an animal immunologically forearmed. But if the CBA cells are injected into a fetal or newborn A-line mouse, they are accepted; more than that, the A-line mouse, when it grows up, will accept any later graft from a CBA donor as if it were its own. I shall begin by using the term *immunological tolerance* in the rather restricted sense that is illustrated by this experiment and shall discuss its more general usage later on.

The experiment I have just described (*1*) can be thought of as an artificial reproduction of an astonishing natural curiosity, the phenomenon of red-cell chimerism in certain dizygotic twins. The blood systems of twin cattle before birth are not sharply distinct from each other, as they are in most other twins; instead, the blood systems make anastomoses with each other, with the result that the twins can indulge in a prolonged exchange of blood before birth. In 1945, R. D. Owen (*2*) made the remarkable discovery that most twin cattle are born with, and may retain throughout life, a stable mixture—not necessarily a fifty-fifty mixture—of each other's red cells; it followed, then, that the twin cattle must have exchanged red-cell precursors, and not merely red cells, in their mutual transfusion before birth. This is the first example of the phenomenon we came to call immunological tolerance; the red cells could not have "adapted" themselves to their strange environment because they were in fact identified as native or foreign by those very antigenic properties which, had an adaptation occurred, must necessarily have been transformed. A few years later R. E. Billingham and I (*3*), with the help of three members of the scientific staff of the Agricultural Research Council, showed that most dizygotic cattle twins would accept skin grafts from each other, and that this mutual tolerance was specific, for skin transplanted from third parties was cast off in the expected fashion. We did not

set out with the idea of studying the immunological consequences of the phenomenon described by Owen; on the contrary, we had been goaded by H. P. Donald into trying to devise a foolproof method of distinguishing monozygotic from dizygotic twins, an enterprise that seemed reasonable enough against the rather thorough background of knowledge we already possessed about the behavior of skin grafts in experimental animals (*4*). It was F. M. Burnet and F. Fenner (*5*) who first read a general significance into Owen's discovery and who wove it into a general hypothesis of the immunological response which counted the phenomenon of tolerance among its theoretical consequences.

In the outcome, it proved impossible to distinguish between the two kinds of twins by skin grafting, but the causal connection between Owen's phenomenon and our own was obvious, and we were now confident of our ability to make adult animals accept tissue homografts by reproducing in the laboratory the very state of affairs that had come about by natural accident in twin cattle. Billingham, L. Brent, and I eventually succeeded in doing this, and our first report on the matter was published in 1953 (*1*). In the meantime, M. Hašek (*6*), working against an entirely different conceptual background, had reproduced Owen's phenomenon in chickens by the ingenious method of making a deliberate synchorial parabiosis between chick embryos in the shell. At hatching the parabionts separated, and from then on they were incapable of making antibodies against each other's red cells, or, as later work showed (*7*), of rejecting grafts of each other's skin. It is now known that chimerism can occur naturally, though rarely, in twin sheep, and more rarely still in twin human beings (*8*); in twin chickens it is

probably the general rule. That chimerism should occur in man is clear proof that the principle of tolerance applies to human beings as well as to laboratory animals, and human chimeric twins can accept grafts of each other's skin (9).

Properties of the Tolerant State

The main points that emerged from our analysis of the tolerant state were these. In the first place, tolerance must be due to an alteration of the host, not to an antigenic adaptation of the grafted cells, for grafts newly transplanted in adult life have no opportunity to adapt themselves, and the descendants of the cells injected into fetal or newborn animals can be shown, by N. A. Mitchison's methods, to retain their antigenic power (10). Once established, the state of tolerance is systemic; if one part of the body will tolerate a foreign graft, so will another; we found no evidence that a tolerated graft builds up a privileged position for itself within its own lymphatic territory. The stimulus that is responsible for instating tolerance is an antigenic stimulus—one which, had it been applied to older animals, would have caused them to become sensitive or immune. A plural stimulus can induce plural tolerance; the donor will usually possess several important antigens that are lacking in the recipient, and long-lasting tolerance must imply tolerance of them all. The state of tolerance is specific in the sense that it will discriminate between one individual and another, for an animal made tolerant of grafts from one individual will not accept grafts from a second individual unrelated to the first; but it will not discriminate between one tissue and another from the same donor. The injection of leucocytes or lymphoid cells can confer tolerance of skin grafts, for example, and later work has shown that the same is true for grafts of thyroid tissue, ovary, kidney, and adrenal gland. These various tissues do indeed differ in their antigenic make-up, but not, apparently, in respect of antigens that play an important part in transplantation immunity. If leucocytes lacked some important antigen present in skin, it is difficult to see how they could confer tolerance of skin, and P. L. Krohn, using more direct and more critical methods, has found it impossible to distinguish between the antigenic make-up, so far as it governs their transplantability, of ovary and skin (11).

Tolerance can be brought promptly and permanently to an end by an experimental device which combines certain principles established by N. A. Mitchison and M. W. Chase. Please cast your mind back to the model experiment I described at the beginning of this article and imagine an A-line mouse which is tolerant of CBA tissue and which carries a CBA skin graft as outward evidence that this is the case. The tolerated CBA graft can be destroyed within a week by injecting into its host cells from A-line mice which have reacted upon and rejected CBA tissues in the expected fashion. A less spectacular but in some ways more informative variant of this "adoptive immunization" is the injection of the tolerant mouse with lymphoid cells from A-line mice that have *not* been sensitized beforehand by CBA tissues. Here too, though much more slowly, the tolerant state is brought to an end. The inference we drew from this experiment —and nothing has occurred since to make us question it—was that tolerance is due to a central failure of the mechanism of immunological response and not to some intercession at a peripheral level.

There is one important question about tolerance which the experimental system I have been describing is very ill equipped to answer: Does the maintenance of the tolerant state depend upon the continuing presence of the antigen that provoked it? When living cells are used to procure tolerance they survive into adult life and therefore maintain a chronic antigenic stimulus, but is it necessary that they should do so? So far as transplantation immunity is concerned, no completely confident answer can be given until the antigens that excite it can be extracted in a sufficiently potent form; but with "tolerance" of foreign proteins and red cells, it does indeed seem that antigen must continue to be present, even though in quantities below the threshold of direct estimation, if a fully nonreactive state is to be maintained (12).

This is one of the most important single pieces of evidence that must be taken into consideration when devising hypotheses to account for tolerance, and it points to the clear distinction that must be drawn between the dosages of antigen necessary, on the one hand, to instate tolerance and, on the other hand, to maintain it. Much else is still uncertain—for example, the significance to be attached to the "partially tolerant"

state. Tolerance is not an all-or-nothing phenomenon; every degree of tolerance is to be found, from that which allows a graft to live just perceptibly longer than would be expected of it in a normal animal to that in which the graft is permanently accepted by and incorporated into its host. Is an animal partially tolerant because all its reactive cells are almost completely debilitated, or because, while most of them are completely out of action, a minority retain full possession of their powers?

Tolerance and Runt Disease

It has been the experience of all laboratories that tolerance is most easily procured by injecting lymphoid cells into immature animals. This is not because lymphoid cells are in themselves more strongly antigenic than others— there is evidence that they are not—but probably because they are distributed throughout the body of the animal into which they are injected. This should make them adept at producing tolerance; an animal can be immune when only a minority of the cells that are competent to react immunologically are doing so, but it cannot be tolerant unless the very great majority of them are not.

Unfortunately, the lymphoid cells used to induce tolerance are immunologically qualified to attack the tissues of their host, with consequences that were first revealed by Billingham and Brent (10) and, in a somewhat different form, by M. Simonsen (13). The injection of foreign adult lymphoid cells into newborn mice of an unrelated strain gives rise to a fatal or chronic illness, "runt disease," marked by retardation of growth and widespread damage to the host's lymphoid tissue. Its discoverers have proved beyond question that runt disease is immunological in origin. So far as it concerns mice, the clear recognition of runt disease had to await the development of a technique for injecting newborn mice intravenously (14); until that had been done, there was every inducement to believe that death or stunting was due to accidental damage caused by injecting cells into mice *in utero*.

Runt disease or splenomegaly (one of its earlier symptoms) provides a test of the immunological competence of cells and lends itself to exact studies of their immunological capabilities; perhaps the most important discovery that can be

credited to it is that of the presence of an immunologically competent cell in peripheral blood. The aspect of runt disease that concerns us here is its relationship to tolerance. A state of tolerance must obviously abet the onset and probably the progress of runt disease, because if adult lymphoid cells are to attack the tissues of the animal into which they are injected they must live long enough to be able to do so. The frequency with which runt disease was associated with the induction of tolerance gave rise to the suspicion that tolerance might itself be a pathological condition. Might not the adult lymphoid cells used to induce it simply exterminate the developing lymphoid cells of the host and take their place? This cannot be so. Tolerance unaccompanied by any symptom of runt disease is produced by the injection of embryonic cells or by a natural or artificial parabiosis between embryos, and it leads in such instances to a stable chimerism in which native and foreign cells seem to coexist without the one ousting the other. It can be produced, moreover, by adult lymphoid cells which, though antigenically foreign to their hosts, are for simple genetic reasons incapable of attacking them. The experiment described at the beginning of this article is in fact best carried out by injecting, not adult CBA lymphoid cells, but adult lymphoid cells from a first-generation hybrid between mice of strains A and CBA. By and large there seems no reason to believe that tolerance can be explained in terms of cellular competition and replacement.

Other Forms of
Immunological Nonreactivity

The antigenic substances I have so far had in mind are "foreign" only in the sense that they derive from other members of their recipients' species. Tolerance of more remotely foreign cellular antigens is more difficult but certainly not impossible to achieve. But even if we confine the concept to the reactions that take place within the compass of a species, it is clear that tolerance can be induced by antigens belonging to more than one chemical class and may extend to more than one modality of response. A tolerant animal not only fails to engage in the "cellular" type of response we associate with the reaction against homografts of skin; it fails equally to make humoral anti-

bodies—for example, isohemagglutinins —and the antibodies that fail to make an appearance include those of so-called "natural" occurrence (for example, human anti-A or anti-B) as well as those that appear only in response to deliberate immunization. It is not surprising, then, that many authors (15) should have found that a state not yet formally distinguishable from tolerance is brought about by the injection of purified protein antigens into young animals.

Even in this wider sense tolerance is by no means the only kind of specific immunological nonreactivity (16). L. D. Felton described and named the phenomenon of "immunological paralysis" in 1949. Felton showed that adult mice could be immunized and therefore protected against otherwise lethal doses of living pneumococci by the injection of 0.5 microgram of a pneumococcal polysaccharide of the appropriate antigenic type. But, far from conferring resistance, the injection of larger doses (500 micrograms, for example) abolished it, for the mice became vulnerable to pneumococcal infection and could no longer be protected against it by an injection of the smaller dose.

An analogous phenomenon was discovered by F. J. Dixon and P. H. Maurer when they showed that large doses of soluble protein antigens failed to excite a response from adult rabbits. The foreign protein disappeared from the circulation at just the rate that would be expected if no antibodies against it had been formed. At one time most of us believed that immunological paralysis could not be classified as an essential nonreactivity. Antibodies were probably formed, we believed, but were promptly bound by antigen present in vast excess. But the most recent work, notably from A. H. Coons's laboratory, makes this interpretation rather doubtful, for refined methods have so far failed to reveal that any immunological reaction is in progress. These observations have given rise to the hope that paralysis and tolerance of soluble antigens are indistinguishable phenomena.

The problem now to be decided is whether or not the inception of tolerance in the narrower sense depends upon some quantitatively distinctive property of immature lymphoid cells. Are embryonic cells specially easy to paralyze, for example, or (this comes to the same thing) is it specially difficult to make adult cells tolerant? I know that

work bearing on this problem is in progress in Coons's and Mitchison's laboratories. For many reasons—one of them the need to distinguish between the doses of antigen needed for the inception and the maintenance of the unresponsive state—the problem is by no means so easy to tackle as might appear at first sight.

It is a matter of some importance that a quantitative study of the same kind should be made of tolerance of foreign tissue grafts. To refer once again to the experiment described at the beginning of this article, we can be sure that an *adult* A-line mouse will not be made tolerant of CBA tissues by the injection into it of a proportionate multiple of the number of CBA cells that would have sufficed if the recipient had been newly born, but we still do not know whether a *dis*proportionately large number of CBA cells might not confer tolerance upon a mouse, say, 10 days old. However, the possibility that tolerance and paralysis are no more than quantitatively distinguishable, if even that, makes it more urgent than ever before to prepare the antigens that excite transplantation immunity in an adequately potent form.

Two other forms of nonreactivity should be mentioned. One is the specific tolerance of tissue grafts which may be conferred upon a totally irradiated mouse whose hematopoietic tissues have been restored by an injection of foreign bone-marrow cells. J. F. Loutit has suggested that the phenomenon is akin to tolerance in the narrower sense defined by our own experiments, but here, too, the analogy with immunological paralysis is not far to seek. Curiously enough, the encouragingly long survival times of kidney homografts reported by our surgical colleagues in Boston and Paris (17) have been brought about by the use of rather lower doses of whole-body irradiation than experience with mice might lead one to regard as essential. Perhaps the behavior of inbred and homozygous mice is not the best theoretical guide to what may be expected of animals so obstinately heterozygous as human beings. Is it possible that "radiation-induced tolerance" may be easier to secure when the mouse donors are hybrids of type X/Y and the recipients hybrids of type Y/Z? This model is also unrealistic, but more akin in principle to the genetic situation that human beings confront us with in real life.

Finally—I have put it last because

it is the least easy to classify, though it might turn out to be the paradigm of all such phenomena—I must mention the inhibition of drug allergy described by M. W. Chase and M. Sulzberger (18). Guinea pigs fed by mouth with, for example, picryl chloride no longer develop a cutaneous hypersensitivity of the delayed type upon the application of that substance to the skin. Analysis has shown that this, too, is an essential nonreactivity. Many of us nourish the hope that a similar principle might be turned to good account in transplantation immunity, if it should prove possible to prepare a "haptenized" derivative of the antigens that cause it to come about.

Biological Significance

Tolerance is a natural phenomenon, though its natural occurrence among chimeric twins may be something of an oddity. Has it any wider zoological significance?

The immunological defenses of the body are directed against foreign matter. It is not at all common for an animal to react upon the native ingredients of its own body. Burnet was the first to realize that this is not a state of affairs to be taken for granted but something that calls for a special explanation, and it was his attempt to explain it that led him to predict that antigens which impinged upon an animal sufficiently early in its life would come to be accepted as if they were its own. Now that tolerance is an established fact we may turn his argument the other way about. Tolerance is ideally well qualified to provide a built-in natural safeguard against the danger that an animal might be sensitized or immunized by the constituents of its own body, and this may turn out to be its biological *raison d'être* (19). If this argument is valid, then the only substances capable of exciting auto-immune reactions should be those which never normally have access to centers of immunological response, so that the body has no opportunity to learn to tolerate them. I do not know of any evidence that contradicts this inference.

Tolerance makes one think anew about the special relationship that holds between a mammalian mother and its unborn young. There are hints in the literature that a mammalian infant may be specially tolerant of antigens of maternal origin, and evidence of such a relationship should be found more readily among human beings than elsewhere, for, unlike rats and mice, we have a long gestation period, and unlike sheep and cattle, our fetal membranes are permeable by at least some molecules as large as proteins. Yet it seems most unlikely that maternally induced tolerance should be of regular or well-defined occurrence. The permeation of the fetus by foreign antigens gaining access through the mother might weaken its resistance in later life to infectious disease (we may recall here the significance read by Burnet and Fenner into Traub's observations on lymphocytic choriomeningitis) (5). This is only one among half a dozen reasons why traffic between maternal and fetal circulations should be under very close control. The occurrence of runt disease makes it of course unthinkable that the barrier between mother and fetus should be easily penetrable by anything so large as maternal cells.

Theories of Tolerance

Burnet and I have agreed upon a division of labor which absolves me from speculating upon the causes of the various kinds of essential nonreactivity I have described. Tolerance, like the secondary response and the nature of immunological "memory," has become something of a testing ground for theories of the immune response. It must be said that the occurrence of tolerance does not yet follow from any hypothesis of the nature of the immune response that has been verified by *independent* means. Far too much is still uncertain. We do not yet know whether any one antibody-forming cell is potentially capable of making any antibody within the organism's immunological repertoire or whether the competence of any one such cell is restricted to a subclass of the reactions that can be engaged in by the organism considered as a whole. We do not yet know whether the act of synthesis undertaken by an antibody-forming cell is strictly and specifically underwritten by the cell's genetic make-up or whether, in J. Lederberg's terminology, the instructions that govern that act of synthesis are imparted by the antigen itself. And if it should be true that the antigen does no more than choose between one set of pre-existing instructions and another, we still do not know whether those instructions are already present in the zygote, and therefore part of the legacy of its descendants, or whether they must be added to (by mutation, necessarily) during the course of growth. Finally, we do not even know whether the antitheses, as I have put them, are wisely put or not. But it is the study of tolerance that has raised these questions in a specially urgent form (16, 20), and that, in due course, will make an important contribution to the answers.

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