AN ANTIBODY RESPONSE TO SKIN HOMOGRAFTS IN MICE

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IT has long been known that the destruction of grafted neoplastic cells of homologous origin is associated with an iso-immune reaction involving the production of circulating antibodies (see review by Hauschka, 1952). It is also generally held that the destruction of skin homografts involves an essentially similar mechanism but, since antibodies have not so far been demonstrated by orthodox serological methods, the possibility exists that orthotopic grafts of normal tissues, such as skin, evoke a somewhat different response from that of malignant cells.

However, Billingham and Sparrow (1954) have now shown that if rabbit skin epithelial cells are suspended in serum from a specifically immunized animal (*i.e.*, one that has received and reacted against skin homografts from the same donor) prior to auto-transplantation, their subsequent growth *in vivo* is partly or completely inhibited. This result is essentially similar to that obtained by Gorer (1942) with leukotic cells in mice and by Kidd (1946) with the Brown-Pearce carcinoma in the rabbit, and may be held to indicate a similarity between the response evoked by various types of cellular homograft, both normal and malignant.

We were further encouraged to reopen the problem of demonstrating an antibody response to skin homotransplants since Gorer and Mikulska (1954) have greatly improved the techniques for detecting iso-haemagglutinins in mice, while Amos (1953), also working with this species, has evolved a method for detecting iso-agglutinins with leucocytes. The particular advantage of the latter technique is that it enables one to detect certain antigens present in some mouse tissues which are not represented in the erythrocytes.

MATERIALS AND METHODS.

The mice used were adult males from highly inbred strains often used in cancer research. The " A " strain is albino with a high incidence of mammary cancer. The C_3H strain is wild type in colour and also has a very high incidence of mammary cancer. The Bagg albino C (usually labelled Balb. C) and the C57 black strains have a low incidence of this disease. Susceptibility to spontaneous tumours is not believed to influence the result of grafting experiments.

Operative methods.—Two types of skin homograft have been used. In the majority of the tests to be described each recipient was given a single full-thickness pinch graft—a disc of full-thickness skin, about 9 mm. in diameter and weighing about 18–20 mg. In others

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the " graft " comprised about 4 small rectangular sheets of the anatomically intact superficial epidermis, prepared by a tryptic digestion technique from thin shavings cut from the skin of the donor's tail. Full details of this technique are given by Billingham and Medawar (1951) who have shown that such grafts are perfectly viable in compatible hosts. Each animal received approximately 1 cm.^{2} of homologous epidermis weighing 6–7 mg.

The grafts, whether full-thickness or of the pure epidermal type, were transplanted to a vascular bed cut to the appropriate size in the skin of the side of the recipient's chest. Full details of the method of preparing the graft bed and the application of dressings are given elsewhere (Billingham and Medawar, 1951). The primary inspection was carried out on the 8th day, and subsequent inspections every 2 days thereafter. When the outward appearance of the grafts was such as to leave no doubt that their breakdown was complete and of long standing (at the 12th–14th day post-operatively) half the animals in each experimental group—which comprised 9–10 mice—were killed with chloroform and bled from the heart ; the serum obtained from their pooled blood was tested for antibodies. The remaining animals in each group were given a second graft from the original donor strain and this regressed at an accelerated rate. Even at the primary inspection of these " second set " homografts, carried out on the 7th day post-operatively, it was apparent that breakdown was already complete. These animals were bled between the 7th and 10th days. Pooled sera were used as before.

Serological methods.—For agglutination of erythrocytes the sera were diluted in saline containing 2 per cent dextran. The most suitable preparation of dextran for this purpose has a mean molecular weight of 100,000 and is prepared as a 10 per cent solution in 6 per cent glucose and autoclaved. The red cells are suspended in suitably absorbed human sera (see Gorer, 1950). In other respects the agglutination technique is the same as that used in *Rhesus* typing.

The leucocytes for the agglutination tests were obtained from the peritoneal washings of mice inoculated 2–5 days previously with *Myco. smegmatis*. Normal saline was used as a vehicle for both cells and antiserum. Equal volumes of each were incubated in small tubes at 37° and the result read microscopically. (Full details of the method are given by Amos, 1953).

Absorption tests are essential in any antigenic analysis, and the ideal absorbing agent would have been a suspension of epidermal cells. Unfortunately the preparation of sufficient quantities of dissociated epithelial cells proved to be technically too exacting in the mouse. Minced whole skin does not form a satisfactory suspension, possibly owing to the preponderance of collagen. Liver can be obtained in adequate amounts, is readily homogenized and has therefore been employed for the majority of absorptions. Absorptions with skin were performed in a few instances but gave no information that was not obtained with liver and will not be further discussed.

During storage at −20° or at room temperature following lyophilization most sera retain their ability to agglutinate red cells for many months, but some appear to develop some substance that strongly inhibits their capacity to agglutinate leucocytes. Fortunately this inhibitor is readily removed by absorption with minced perfused liver of the strain in which the antibodies were formed and this absorption was performed in all the tests with leucocytes to be described.

RESULTS.

The Antibody Response of C57 Blacks to Full-thickness Skin Homografts from Balb. C.

Table I*a* shows the titres of haemagglutinin demonstrable after the animals had reacted against either a single or two successive skin homografts. It will be seen that in addition to the red cells of the donor strain, those of the A strain were also agglutinated. It is perhaps surprising that the titre against the latter strain is higher in each case. We cannot discuss the matter fully here, but the available evidence suggests that we are dealing with a single antigen common to the red cells (and fixed tissues) of both strains, and that the A strain red cells are peculiarly susceptible to iso-agglutination.

TABLE I*a*.—*Antibody Response of C*57 *Blacks to Full-thickness Skin Homografts from Balb. C* (*Red Cell Agglutinins*).

Red cells of strain :	A.	Balb.C.	C57.	C_3H.
After first graft . .	64	16	0	0
„ second „ . .	1024	128	0	0

Titres in this and succeeding tables expressed as reciprocals

TABLE I*b*.—*Antibody Response of C*57 *Blacks to Full-thickness Skin Homografts from Balb. C* (*Leucocyte Agglutinins*) *after Second Graft.*

Leucocytes of strain :		A.	Balb. C.	C57.	C_3H.
Absorbed with liver from strain :	C57 . .	32	64	0	8
	A . .	0	32	0	0
	Balb. C .	0	0	0	0
	C_3H .	8	32	0	0

Table I*b* shows the iso-agglutinin titres determined by the leucocyte agglutination method, which are somewhat different. Following the control absorption the leucocytes of the C_3H strain were agglutinated, albeit to a lower titre than those of strains A and Balb. C. We may also point out that the peculiar sensitivity of A red cells is not shown by their leucocytes. As was expected the liver from Balb. C removed all agglutinins. However, A strain liver failed to remove all antibodies for the donor strain and we thus have evidence for the formation of a second antibody. In addition the feeble agglutinin acting on the C_3H leucocytes may indicate the presence of a third antibody. However, it may be that C_3H cells have an antigen very similar to that in the A strain. The result of absorption with C_3H liver giving a significant reduction of titre for A leucocytes is consistent with the latter hypothesis. The lowering of the titre for Balb. C cells is probaby non-specific.

At present it seems justifiable to conclude that red cell agglutination shows the formation of an antibody to one antigen, whilst the leucocyte agglutination enables at least one other factor to be identified.

*The Antibody Response of C*57 *Blacks to Pure Epidermal Homografts from Balb. C.*

Table II shows the primary and secondary responses for red cell agglutinins elicited by homografts of this type. The differences in titre following pure epidermal homografting, and following full-thickness skin grafting are within the range of experimental error. The results show that the dermal component of a skin homograft plays no essential rôle in the stimulation of the immune response and may be of minor importance in determining its magnitude.

TABLE II.—*Antibody Response of C*57 *Blacks to Pure Epidermal Homografts from Balb. C* (*Red Cell Agglutinins*).

Red cells of strain :	A.	Balb. C.	C57.	C_3H.
After first graft . .	128	32	0	0
„ second „ . .	512	128	0	0

A similar conclusion was reached by Billingham and Sparrow (1954) who have shown that the median survival time of pure epidermal homografts in the rabbit is only very slightly greater than that of full-thickness skin homografts of comparable surface area.

The Antibody Response of C_3H Mice to Full-thickness Skin Homografts from A Strain Mice.

Table III*a* indicates that in so far as red cell agglutination is concerned the antibody response of C_3H mice to A strain skin grafts is very similar to that already shown for C57 mice to Balb. C skin. However, Gorer and Mikulska (unpublished) have shown that if C_3H mice are hyperimmunized with an A strain mammary tumour an agglutinin appears that reacts with the red cells of C57 blacks. This brings the result of red cell agglutination in line with those shown for leucocytes in Table III*b*. Here it will be seen that the leucocytes of C57 blacks are agglutinated. The livers of both A and Balb. C remove all antibodies, whilst those of the C57 blacks remove only their own agglutinin (the lowering of titre for A strain leucocytes is probably not significant). In this case it will be seen that we have evidence of only two antibodies being formed, both the corresponding antigens probably being common to red cells and other tissues.

TABLE III*a*.—*Antibody Response of C_3H Mice to Full-thickness Skin Homografts from A Strain (Red Cell Agglutinins).*

Red cells of strain :	A.	Balb. C.	C57.	C_3H.
After first graft	128	32	0	0
„ second „	256	128	0	0

TABLE III*b*.—*Antibody Response of C_3H Mice to Full-thickness Skin Homografts from A Strain (Leucocyte Agglutinins) after Second Graft.*

Leucocytes of strain :		A.	Balb. C.	C57.	C_3H.
Absorbed with liver from strain :	A	0	0	0	0
	Balb. C.	0	0	0	0
	C57	32	64	0	0
	C_3H	64	64	16	0

In Table IV we have summarized our findings regarding graft dosage and antibody response ; for the latter the figures are given for the donor strain only. The difference between the primary response (that following a single homograft) and the secondary response (that following a second homograft after regression of the first) may to some extent be influenced by variations in experimental procedure. In any case it is not known what relationship exists between the

TABLE IV.—*Summary of Results.*

Donor strain.	Recipient strain.	Nature of graft.	Number of immunizations.	Interval between grafts (days)	Interval between operation and bleeding (days)	Number of mice.	Red cell titre.	Leucocyte titre.
1. Strong A	C_3H	Full-thickness	1	—	15	4	16	16
	„	„	2	21	9	4	256	64
2. Strong A	„	„	1	—	13	3	128	—
	„	„	2	18	8	4	256	32
3. Balb. C	C57	„	1	—	12	5	16	—
	„	„	2	17	8	5	128	32
4. Balb. C	„	Pure epidermal	1	—	15	3	32	32
	„	„	2	19	9	4	128	32

amount of antibody and the agglutination titre. A more adequate method of measuring the former might be to measure the amount of any tissue, such as the liver, needed to remove all detectable antibody.

DISCUSSION.

Our knowledge of iso-immune reactions has been almost entirely limited to reactions observable with red cells. It is a fortunate fact that some of the important antigens in the mouse are shared by its red cells and fixed tissue cells. Medawar's (1946) studies show that this is probably not so in the rabbit, and this goes far to explain the delay in detecting antibodies to skin homografts in this species. We are at present engaged in studying the ability of mouse erythrocytes to stimulate an immune response against skin homografts.

Even with red cells special methods have often to be used in order to bring about their agglutination or haemolysis. When this is so we speak of incomplete antibodies. This may well be a misnomer. For example, in mice we may have to use the human serum : dextran system to detect an antigen on the red cells, while the same antigen on the leucocytes may be detectable in a saline medium, in which case the corresponding antibodies are " complete." Whether we refer to these antibodies as " incomplete " or " inconvenient " it is obviously desirable to improve our methods of detecting those reacting with cells other than erythrocytes. Our knowledge of the rôle played by antibodies in the destruction of homografts is very meagre. The experiments quoted in the introduction show that antibodies have some direct deleterious effect *in vivo*, but it remains to be shown why they are apparently inactive in tissue culture (see Medawar, 1948 ; Allgöwer, Blocker and Engley, 1952 ; Amos, 1953) and why passive immunity to homologous tissue grafts has never been transmitted by means of serum (Mitchison, 1953 ; Billingham, Brent and Medawar, unpublished.)

The primary object of the present paper was to show that antibodies are formed in response to orthotopically transplanted skin homografts. A thorough antigenic analysis is a very involved process but the limited data presented here point to certain facts that may prove of clinical importance in homografting in man. The small number of antibodies that are detectable does not reflect the actual number of antigens that may be confidently assumed to differentiate the skins of any two mouse strains. Bittner (1936) made a genetic study of splenic homografts in mice from which one may deduce that strains may differ by seven or more antigens. Medawar (1945), studying skin homografts in rabbits and using a different approach from that of Bittner, gave seven as the minimum number of antigens likely to differentiate two unrelated rabbits. The fact that less than half this number of antibodies have been demonstrated in any given mouse serum indicates that some antigens are far more potent stimuli than others. This indeed is well known in human iso-immunization. According to figures given by Race (Mollison, Mourant and Race, 1952) anti-C is only present in about 30 per cent of sera resulting from Rh. immunization ; in theory it could be formed in about 80 per cent but apparently anti-D is usually formed preferentially. It is very probable that such differences in antigenic potency not only influence the titres observed *in vitro* but the fate of grafts *in vivo*. Thus working with a mouse sarcoma Gorer (1938) showed that if mice were compatible for a major antigen, a graft might persist for three months or more, whilst if they were not so it

regressed in about two weeks. It is therefore possible that we might greatly prolong the life of human homografts if we could match them for a relatively small number of major antigens. It would very seldom be possible to obtain compatibility for all of a very large number of antigens.

SUMMARY.

Incompatible skin homografts in mice stimulate the formation of antibodies that react with red cells and leucocytes. Some of the pertinent antigens may be absent from the red cells. All are apparently present in the liver and in leucocytes.

Homografts comprising the superficial epidermis freed from all dermal elements are apparently as effective in stimulating antibody production as are full-thickness grafts.

It is pointed out that not all antigens are equally potent, and some antibodies are apparently formed preferentially. This may prove of clinical importance.

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