# AN ANALYSIS OF GRAFT-VERSUS-HOST DISEASE IN SYRIAN HAMSTERS

## II. The Epidermolytic Syndrome: Studies on Its Pathogenesis*

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(Received for publication 13 February 1970)

In the preceding report (1), a lethal form of graft–versus–host (GVH) disease was described in adult Syrian hamsters in which a severe, cutaneous, inflammatory reaction culminating in epidermolysis was the hallmark. Various findings indicated that this extensive epidermolysis was not explicable on the basis of a direct immunological attack by grafted donor lymphoid cells upon host epidermal cells confronting them with homologous host transplantation antigens. It was suggested, as an alternative hypothesis, that dissolution of the union between dermis and epidermis might be attributable to an autoimmune attack arising from the intense cutaneous inflammatory reactions incited by the local inoculation of specifically sensitized donor lymphoid cells. The resultant cutaneous inflammation might have unwittingly exposed important, normally sequestered, epidermal–specific antigens which incited lymphoid cells of either donor or host origin to an immune response directed against the skin components concerned, thereby producing the characteristic, progressive generalized epidermal exfoliation.

The idea that autoimmune responses may arise *indirectly* from intense inflammatory reactions is not a new one (2), though it remains a concept with little supportive evidence. The experiments reported herein were designed to evaluate this possibility.

### *Materials and Methods*

Both the hamsters employed and most of the methods in the present experiments have been described in detail in the preceeding communication (1).

Inoculation of Cell Suspensions by other than the intracutaneous route were carried out as follows:

*Intravenous.*—A volume not exceeding 0.5 ml. of cell suspension was introduced through a No. 30 gauge needle into a femoral vein.

*Intraperitoneal.*—Cell suspensions up to 1.0 ml. in volume were delivered directly into the peritoneal cavity via a No. 23 gauge needle.

* This work was supported by U. S. Public Health Service Grant AI 07001 and by a Pennsylvania Plan Award.

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*Intramuscular.*—The required number of cells suspended in a volume of 0.5 ml. were injected in 0.1 ml. aliquots via a No. 25 gauge needle at different sites in the hamstring and dorso-lumbar muscles.

Horse anti-hamster lymphocyte serum (ALS), kindly provided by Dr. C. F. Shaffer, was prepared by injecting a horse subcutaneously with $200 \times 10^6$ hamster lymph node cells suspended in Complete Freund's Adjuvant, followed in a month by four additional intravenous boosts of $1 \times 10^9$ hamster lymphoid cells spaced at 1 wk intervals. 1 wk later the serum was harvested and, when administered at a dose schedule of 1.0 ml. twice a week, was capable of sustaining homografts of skin beyond 50 days on treated hamsters (3).

MHA donors of the bone marrow cells used ig the experiments described on page 189 were injected intramuscularly with 1 ml. of ALS on days 7, 4, and 1 before their sacrifice.

## EXPERIMENTS AND RESULTS

*Influence of Route of Inoculation on Elicitation of Epidermolysis.*—Even before the possibility was considered that the epidermolysis might have an autoimmune basis, several lines of evidence pointed to the importance of the *skin* in the elicitation of this syndrome in hamsters. Most pertinent was the observation that this unique accompaniment of graft-versus-host reactivity was initiated consistently by inoculation of donor lymphoid cells into the skins of $F_1$ hybrids. To define further the importance of this route of inoculation, parallel experiments were set up in which panels of 10 or more adult (CB $\times$ MHA)$F_1$ hybrids received a standard inoculum of $200 \times 10^6$ MHA-anti-CB lymphoid cells by one of the following routes: intracutaneous, intramuscular, intraperitoneal, and intravenous. The results of these experiments (see Table I) indicate that epidermolysis could only be initiated by the administration of donor cells by the *intracutaneous* route. The utter dependence of the pathogenesis of this condition upon cutaneous inoculation was surprising since homologous diseases have been readily procurable in other species by employing the intravenous or intraperitoneal routes (4). It should be pointed out that $F_1$ hybrid recipients of MHA lymphoid cells introduced by parenteral routes *other* than the skin ultimately exhibited a more classic picture of chronic homologous disease of greatly delayed onset and devoid of the epidermolytic component. This will form the basis of a subsequent report.

It has previously been reported that the acute skin disorder is transferable adoptively from affected to normal $F_1$ hybrids by means of viable lymphoid cells (1). In the relevant experiments, both the inciting inoculum of MHA-anti-CB cells, and the "transferred" lymph node cells were delivered to their respective hosts by the intracutaneous route. In light of the apparent requirement for *intracutaneous* inoculation of putative attacking cells, tests were performed to determine whether passage of the disease from affected to normal animals was also dependent upon intracutaneous injection of cells. Two panels of 10 (CB $\times$ MHA)$F_1$ hybrids were challenged with $200 \times 10^6$ MHA-anti-CB lymphoid cells per host, the animals in panel 1 receiving the donor cell inoculum

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via their skins, while those in panel 2 received similar cells by the intravenous route. 7 days later both groups of animals were killed, their lymph nodes pooled, and cell suspensions prepared therefrom. One donor equivalent of cells from a panel 1 donor was delivered to each of 10 normal (CB × MHA)$F_1$ hybrid hosts intravenously; whereas the lymph node cells harvested from panel 2 animals were given to a second group of 10 normal (CB × MHA)$F_1$ hybrids (again 1 donor equivalent per host) by the intracutaneous route (see Table II). The development of epidermolysis in the secondary hosts was found to be *completely* dependent upon the route of inoculation by which the *primary* host had received the MHA cells. If these attacking cells were given initially by vein, subsequent transfer to secondary hosts by the intracutaneous route failed to evoke epidermolysis. However, if the attacking cells reached their initial host through

TABLE I

*Influence of Route of Inoculation of 200 × $10^6$ MHA–anti-CB Lymph Node Cells on Elicitation of Epidermolysis in (CB × MHA)$F_1$ Hybrids*

| Route of inoculation | No. of animals tested | No. of animals | | | |
|---|---|---|---|---|---|
| | | With epidermolysis | | Alive at 30 days | |
| | | | % | | % |
| Intracutaneous | 50 | 50 | (100) | 1 | (2) |
| Intravenous | 50 | 0 | (0) | 48 | (96) |
| Intraperitoneal | 15 | 0 | (0) | 15 | (100) |
| Intramuscular* | 16 | 0 | (0) | 16 | (100) |

* Aliquots of the total dose of donor cells were delivered into multiple sites in hamstring and dorso-lumbar muscle groups.

the skin, the disease could be transferred successfully to secondary $F_1$ hosts by the intravenous route, i.e., *without* a renewed exposure to the cutaneous milieu.

To investigate further the apparent importance of the cutaneous route, (CB × MHA)$F_1$ hybrid hosts were challenged with a standard dose of 200 × $10^6$ MHA–anti-CB lymphoid cells divided so that a variable proportion was delivered intracutaneously, while the remainder was given intravenously. The results are presented in Table III Since prior experiments had indicated that 50 × $10^6$ MHA–anti-CB lymphoid cells given intracutaneously would elicit epidermolysis in adult $F_1$ hybrids, it was not surprising that a similar dose given in conjunction with an additional 150 × $10^6$ MHA cells delivered intravenously would also evoke the acute disease. However, it was unexpected that as few as 20 × $10^6$ MHA cells administered intracutaneously along with 180 × $10^6$ similar cells given intravenouly regularly incited the acute skin syndrome, and in some instances as few as 10 × $10^6$ cells delivered via the former route were effective when combined with the intravenous injection. Neither of these in-

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tracutaneous inocula when administered *alone* to adult $F_1$ hybrid hosts evoked the disease.

*Effect of Excision of Inoculation Sites on Development of Epidermolysis.*— Previous observations on epidermolysis in $F_1$ hamsters suggested that the dermal-epidermal cleavage began at the inoculation sites and spread centrifugally until the entire integument was affected. It seemed as if the inflammatory re-

TABLE II

*Influence of Route of Inoculation on Inciting Cells on Adoptive Transfer of Epidermolysis in Hamsters*

Each primary (CB × MHA)$F_1$ host received 200 × $10^6$ lymphoid cells from MHA–anti-CB donors. All secondary hosts were (CB × MHA)$F_1$ hybrids, which received one donor equivalent of lymph node cells from appropriate primary hosts.

| Route of inoculation into primary hosts | Route of inoculation into secondary hosts | No. of secondary hosts tested | No. of secondary hosts exhibiting epidermolysis |
|---|---|---|---|
| Intracutaneous | Intravenous | 10 | 9 |
| Intravenous | Intracutaneous | 8 | 0 |

TABLE III

*Capacity of Suspensions of MHA–anti-CB Lymph Node Cells to Elicit Epidermolysis in (CB × MHA)$F_1$ Hybrid Hamsters Inoculated by Intravenous and/or Intracutaneous Routes*

| No. of donor cells injected (× $10^6$): | | Appearance of epidermolysis | | |
|---|---|---|---|---|
| Intravenously | Intracutaneously | No. of hosts tested | No. with epidermolysis | |
| | | | | % |
| 200 | 0 | 50 | 0 | (0) |
| 190 | 10 | 6 | 2 | (33) |
| 180 | 20 | 8 | 7 | (87) |
| 150 | 50 | 6 | 6 | (100) |
| 100 | 100 | 6 | 6 | (100) |
| 20 | 180 | 6 | 6 | (100) |
| 0 | 200 | 50 | 50 | (100) |
| 0 | 50 | 13 | 13 | (100) |
| 0 | 20 | 12 | 0 | (0) |
| 0 | 10 | 6 | 0 | (0) |

actions that developed at the injection sites acted as "infecting" foci from which the generalized cutaneous disease evolved. To test this hypothesis, a group of (CB × MHA)$F_1$ hybrids each received 10 aliquots of 20 × $10^6$ MHA–anti-CB lymph node cells injected into their dorsal skins at separate sites. After 1, 2, 3, and 4 days had elapsed, subgroups of these animals were subjected to wide excision of their inoculation sites, down to and including the panniculus carnosus muscle. They were then observed for the development of epidermolysis (Table

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IV). The results were incisive: excision of all immune lymphocyte transfer reaction sites 24 hr postinoculation prevented the subjects from developing any signs of the acute skin disorder. However, when excision of lesions was delayed 2, 3, or 4 days, all of the animals developed severe epidermolysis and succumbed.

To complement the studies described above, experiments were conducted in which cutaneous inoculation sites excised from $F_1$ hybrids challenged intracutaneously with MHA lymphoid cells were tested for the presence of cells capable of causing the disease when administered to normal $F_1$ hybrids; i.e., the ability to transfer the disease adoptively. Two approaches were employed: the pieces of excised skin, which included the ILT[1] reaction sites, were either (*a*) grafted directly to prepared beds on normal or sublethally irradiated (CB × MHA)$F_1$ hybrid hosts, or (*b*) minced finely, and teased apart to liberate suspensions of

TABLE IV

*Influence of Excision of Immune Lymphocyte Transfer Reactions on Appearance of Epidermolysis in (CB × MHA)$F_1$ Hybrids Challenged Intracutaneously with MHA-anti-CB Lymph Node Cells*

| Time after inoculation of excision of cutaneous lesions | No. of animals tested | No. of animals with epidermolysis | |
|---|---|---|---|
| *days* | | | % |
| 1 | 12 | 0 | (0) |
| 2 | 10 | 9 | (90) |
| 3 | 10 | 10 | (100) |
| 4 | 6 | 6 | (100) |

round cells which were then harvested and injected into the skins of normal or sublethally irradiated hybrid hosts. These studies were performed with cells from lesions of 24, 48, and 72 hr standing. No signs of epidermolysis developed in normal $F_1$ hybrids that received lesion material by either procedure. However, clear-cut evidence of epidermolysis was forthcoming in three of four sublethally irradiated hosts receiving cells from "teased" lesions and in four of six similar hosts grafted with skin removed from primary hosts 24 hr after MHA lymphoid cell challenge. By contrast, neither grafts of intact skin nor "teased" cells obtained from skin reaction sites removed from primary $F_1$ hosts 48 or more hours after initial challenge were able to incite the disease.

All the evidence so far presented implies that, to evoke acute epidermolysis in $F_1$ hybrid hamsters, the specifically sensitized parental strain lymphoid cells *must* be injected *directly* into the host skin; it also suggests that, having remained at the local sites for about 24 hr, the attacking cells and/or their progeny emigrate in sufficiently large numbers that subsequent removal of the original skin lesions fails to influence the course of the impending disease. These results would seem to rule out the possibility that the original lesions acted as impor-

[1] ILT, immune lymphocyte transfer.

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tant "infecting" foci from which a generalized epidermolysis could proceed by direct extension. Lastly, the results of the various experiments reported strongly support the hypothesis that an autoimmune process directed against the epidermis may be the proximate cause of epidermolysis in hamsters.

*Experimental Reconstitution of Hypothetical Inflammatory Reaction in the Presence of Epidermal Antigens.*—To provide the clinching piece of evidence needed to validate the autoimmune hypothesis, it was necessary to reconstruct, by experimental artifice, the postulated confrontation between attacking MHA lymphoid cell and CB transplantation antigens in a milieu containing the puta-

TABLE V

*Capacity of Various Cellular Mixtures to Elicit Epidermolysis following Intramuscular Inoculation into (CB × MHA)$F_1$ Hybrids*

| Contents of cellular inoculum | No. of cells per host ($\times 10^6$) | No. of animals tested | No. which developed epidermolysis |
|---|---|---|---|
| MHA–anti-CB lymph node | 200 | 10 | 10 |
| + | | | |
| CB epidermal | 50 | | |
| MHA–anti-CB lymph node | 200 | 6 | 6 |
| + | | | |
| MHA epidermal | 50 | | |
| + | | | |
| (CB × MHA)$F_1$ lymph node | 120 | | |
| MHA–anti-CB lymph node | 200 | 10 | 9 |
| + | | | |
| (CB × MHA)$F_1$ lymph node | 120 | | |
| MHA–anti-CB lymph node | 200 | 16 | 0 |
| CB epidermal | 50 | 4 | 0 |
| (CB × MHA)$F_1$ lymph node | 120 | 4 | 0 |

tive skin-specific antigens, and in some site other than the skin. To accomplish this, suspensions of specifically sensitized cells from MHA strain donors were mixed with suspensions of epidermal cells prepared from normal CB donors and inoculated intramuscularly in (CB × MHA)$F_1$ hybrid hamsters. It had previously been shown that $200 \times 10^6$ MHA–anti-CB lymphoid cells inoculated intramuscularly into $F_1$ hamsters failed to provoke the acute skin disease (1). In the current experiments, however, it was found that when these cells were injected IM in conjunction with $50 \times 10^6$ CB epidermal cells into $F_1$ hybrid hosts, these animals developed full-blown epidermal necrolysis and most died. Inoculation of $50 \times 10^6$ CB epidermal cells in the absence of admixed MHA node cells failed to affect the well-being of $F_1$ hybrid recipients (Table V).

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In a variant of the above experiment, mixtures were prepared containing MHA–anti-CB lymphoid cells, MHA epidermal cells, and (CB × MHA)$F_1$ lymph node cells as sources of CB transplantation antigens. These mixtures, upon intramuscular inoculation into normal (CB × MHA)$F_1$ hybrids, also caused the acute cutaneous disease. In this experiment it was presumed that the initial immunologic event was the attack upon $F_1$ lymph node cells by MHA lymphoid cells, and that in the inflammatory reaction that ensued, previously hidden antigenic determinants on the "disinterested" MHA epidermal cells were exposed, leading to a second-order immune response directed against these very same epidermal-specific antigens.

In the control of these experiments, cell mixtures comprising only MHA–anti-CB lymphoid cells and (CB × MHA)$F_1$ lymphoid cells were injected intramuscularly into adult $F_1$ hybrid hamsters. Quite unexpectedly these $F_1$ hybrids that received homologous lymphoid cell mixtures, *devoid* of epidermal cells, also developed severe epidermolysis and died (Table V). Clearly, no interaction could conceivably have taken place between immunologically competent cells and epidermal-specific antigens at the inoculation sites in the muscles of these animals.

Similar conclusions were suggested by a different experiment. Previous studies had shown that: (*a*) a standard inoculum of $200 \times 10^6$ MHA–anti-CB lymphoid cells administered intravenously to (CB × MHA)$F_1$ hybrids failed to evoke epidermolysis, and (*b*) exposure of an $F_1$ hybrid to sublethal irradiation significantly lowered the threshold by which a subsequent dose of MHA–anti-CB lymphoid cells could cause the disease when injected intracutaneously. Based on these findings, a group of 10 (CB × MHA)$F_1$ hybrid hamsters was irradiated with 300 r and, 24 hr later, given $200 \times 10^6$ MHA–anti-CB lymphoid cells intravenously. Once again, in an experimental situation in which there seemed little possibility for an immunologic interaction to develop within the confines of the skin, all 10 of these animals developed the typical signs of the acute skin syndrome.

*Reconsideration of the Passenger Cell Hypothesis.*—Having discarded the autoimmune hypothesis, another explanation was sought. Assuming that the sequence of events culminating in epidermolysis was initiated by the immunological attack of the grafted cells against host antigen-bearing cells of some kind, an earlier hypothesis was reconsidered: (*a*) host peripheral blood leukocytes, which normally percolate through the skin and subcutaneous tissues, may provide sufficient antigenic stimulus in their own right, and (*b*) that an immune response directed at these cells could indiscriminantly injure "innocent bystander" cells—epidermal cells in this instance—thereby provoking epidermolysis. The experiments described below were designed to evaluate this possibility.

*Demonstration of Rapidity with which Leukocytes Populate the Skin.*—

Survival of skin isografts and homografts on $F_1$ hamsters with epidermolysis:

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10 (CB × MHA)$F_1$ hybrids received intracutaneous inocula of 200 × $10^6$ MHA–anti-CB lymphoid cells, immediately followed by full thickness grafts of (CB × MHA)$F_1$ and MHA skin transplanted on opposite sides of the thorax. Inspection of these grafts was carried out at 3 and 5 days, and at each subsequent day. Since the inocula of donor cells were in the supralethal range, each of these animals followed a typical clinical course, developing local epidermolysis at 7 days, followed by generalized involvement of the skin within the next 3 days. The grafts of $F_1$ and MHA skin which had healed in and acquired a vigorous blood supply by the 5th day after grafting, suddenly exhibited complete epidermolysis and underwent total rejection between the 10th and 11th day, i.e., the grafts in their entirety were sloughed. Although the interpretation of this finding does not differ from that of previous experimental results where epidermolysis extended through established isografts, the present observations do indicate that, if passenger leukocytes in extravascular spaces are responsible for the antigenic stimulus in epidermolysis, they rapidly attain threshold concentration in the skin, within 1 wk in freshly grafted skin.

Detection of chimerism in MHA hosts reconstituted with $F_1$ cells: A somewhat different means of assessing the speed with which extravascular host leukocytes may contribute to cutaneous antigenicity involved the use of immune lymphocyte transfer (ILT) reactions (5) in lethally irradiated MHA hosts that had been reconstituted with a mixture of lymph node and bone marrow cells from (CB × MHA)$F_1$ hybrid donors. Panels of such reconstituted MHA hosts, putatively chimeric with respect to $F_1$ leukocytes, were challenged intracutaneously with standard inocula of 10 × $10^6$ MHA–anti-CB lymphoid cells at 1, 2, 4, 6, 8, 10, and 12 days after reconstitution. The skin test sites were read at 24 hourly intervals and delayed inflammatory reactions were taken as indicative of the existence of CB transplantation antigens, presumably present on blood-borne $F_1$ hybrid leukocytes at the skin test site. Even at the earliest testing period, i.e. 24 hr after administration of (CB × MHA)$F_1$ lymphohematopoietic cells to lethally irradiated MHA hamsters, positive ILT reactions consistently developed. In fact, the intensity of these reactions was not less than that of similar reactions evoked by challenge at 7 or even 12 days after reconstitution. This evidence suggests that within 24 hr of administration of $F_1$ lymphohematopoietic cells, the latter (or their progeny) enter the blood stream whence they migrate into tissue sites in sufficient number to be recognized antigenically. It should be mentioned that as a consequence of performing these skin tests, in the course of which each animal received an aggregate dosage of 50–100 × $10^6$ sensitized cells, all the chimeric hosts developed severe epidermolysis.

*Susceptibility to Epidermolysis of $F_1$ Hamsters Chimeric with Respect to Parental Leukocytes.*—In the preceding experiments, despite the genetic identity of putative attacking cells and their *host*, these animals developed the acute skin disease. Although these findings suggest that passenger leukocytes are sufficient

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in their own right as antigenic stimuli for the procurement of epidermolysis, a more stringent theoretical requirement could be met by answering the corollary: are passenger leukocytes the *only* important source of antigen necessary for producing epidermolysis in hamsters? It seemed that the ideal experimental animals for an analysis of this question would be (CB × MHA)$F_1$ hybrid hamsters whose own circulating leukocytes of myeloid and lymphoid origin had been replaced by cells derived from MHA donors. Should passenger cells be the only important source of antigen in epidermolytic disease, then these animals should

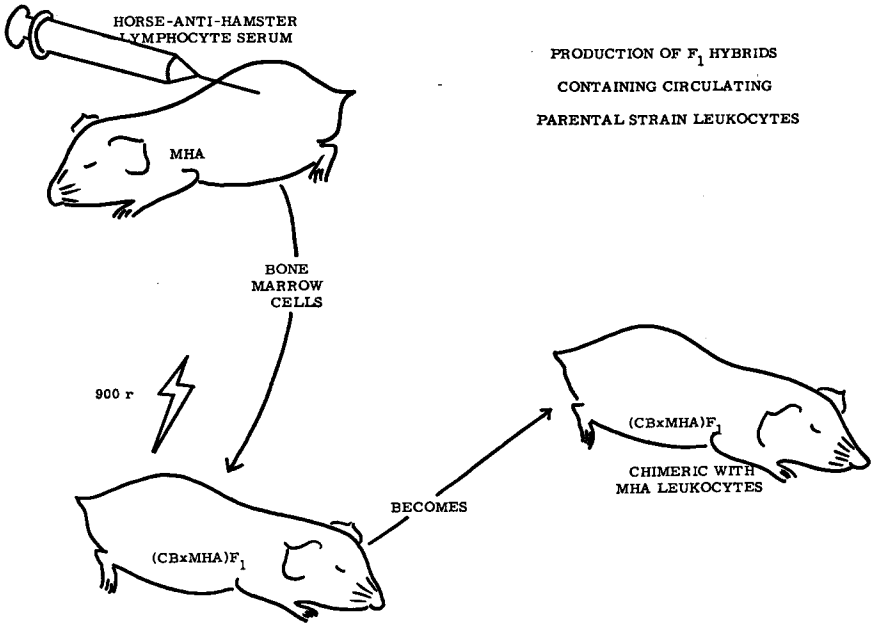


FIG. 1. (CB × MHA)$F_1$ hybrid hamsters were rendered chimeric with respect to MHA lymphohematopoietic cells in this fashion. These animals served as hosts for experiments bearing on the role of "passenger" leukocytes in the pathogenesis of epidermolysis.

be resistant to subsequent intracutaneous challenge with MHA–anti-CB lymphoid cells.

Unfortunately, initial attempts to produce $F_1$ hybrids chimeric with respect to MHA leukocytes met expected failure. Lethal irradiation of $F_1$ hybrids followed by the intravenous inoculation of bone marrow cells from normal MHA donors produced a typical form of postirradiation or "secondary" disease to which these host animals succumbed 15 to 21 days after irradiation. Clearly, such animals could not serve as the experimental subjects in the studies suggested above. This complication was circumvented by making use of the ob-

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servation that bone marrow cells derived from MHA donors which had been treated with ALS were capable of effectively rehabilitating $F_1$ hybrids exposed to lethal doses of whole body X-irradiation; more important, these restored and now chimeric hamsters developed no sign of graft-versus-host disease whatever —specifically, no evidence of epidermolysis. Accordingly, a large group of (CB × MHA)$F_1$ hybrids were exposed to 900 r and reconstituted within 24 hr with one donor equivalent each of bone marrow cells from ALS-treated MHA hamsters (Fig. 1). These animals served as test subjects for the experiments now to be described. Beginning at the 2nd wk after irradiation, subgroups of these animals were studied at 2 weekly intervals in the following manner: at each test period four hybrid chimeras were inoculated intradermally with 200 × $10^6$ MHA–anti-CB lymph node cells, and another four received a similar number of

TABLE VI

*Results of Experiments in (CB × MHA)$F_1$ Hybrid Hamsters Irradiated with 300 r and Reconstituted with Hematopoietic Cells from MHA Donors Treated with Horse-anti-Hamster Lymphocyte Serum*

| Time after reconstitution with MHA-ALS bone marrow | Intensities of ILT reactions incited by lymph node cells from | | Intensities of direct reaction incited by lymph node cells from chimeric-$F_1$ donors in MHA–anti-CB hosts | Appearance of epidermolysis in chimeric-$F_1$ hosts incited by lymph node cells from | |
|---|---|---|---|---|---|
| | CB–anti-MHA donors | MHA–anti-CB donors | | CB–anti-MHA donors | MHA–anti-CB donors |
| *wk* | *10 × $10^6$ cells/inoculum* | | | *200 × $10^6$ cells/host* | |
| 2 | 2.5+* | 2.3+* | 2.0, 2.0, 1.5, 1.5 | 4/4 | 4/4 |
| 4 | 2.2 | 1.4+ | 0, 0, 0, 0 | 4/4 | 4/4 |
| 6 | 2.7+ | 0.4+ | 0, 0, 0, | 3/3 | 0/4 |
| 8 | 2.8+ | 1.3+ | 1.3, 0, 0 | 4/4 | 2/4 |
| 10 | Not done | 1.8+ | 1.5, 1.3 | 4/4 | 2/4 |
| 12 | 2.0+ | 1.6+ | | 4/4 | 4/4 |

* Mean peak reaction score of at least 20 cutaneous inoculation sites. Only scores of > 1+ are considered to be significantly positive.

CB–anti-MHA cells. The inoculation sites were inspected at 24 hourly intervals and scored as conventional ILT reactions. The animals were then observed for periods up to 3 months for evidence of epidermolysis and/or other signs of systemic graft-versus-host disease. Additional hybrid chimeras were sacrificed at each test period, their lymph nodes removed, and cell suspensions prepared from them inoculated into the skins of MHA hamsters preimmunized to CB transplantation antigens to test for the presence of CB antigens, and by implication of CB cells in the inoculum. Finally, skin grafts were removed from these same hybrid chimeras and transplanted to normal MHA animals to determine their survival times.

These experiments and their results are summarized in Table VI. Examina-

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tion of the ILT reaction data shows that the capacity of CB–anti-MHA lymphoid cells to incite significantly positive reactions in the skins of hybrid chimeras remained undiminished throughout the experimental period. This was in sharp contrast to the capacity of MHA–anti-CB cells injected under the same conditions. By as early as the 4th wk after reconstitution, MHA cells were able to mount only feeble ILT reactions in hybrid chimeras, and by the 6th wk no inflammatory reactions whatsoever appeared at the sites of cellular inoculation.

Direct reactions carried out with lymph node cells derived from hybrid chimeras to determine whether CB antigens could be detected therein were significantly positive indicating the presence of CB tissue antigens 2 wk after reconstitution. They became negative when repeated at 4 wk and remained so until beyond the 8th wk after reconstitution.

The ILT reactions evoked by CB–anti-MHA node cells regularly gave rise to severe lethal epidermolysis in hybrid chimeric hosts at each test period. By contrast, MHA–anti-CB node cell inocula were able to effect epidermal necrolysis in hybrid chimeras only at 2 to 4 wk after reconstitution. By 6 wk, these chimeric $F_1$ animals were impervious to the induction of epidermolysis by specifically sensitized MHA lymphocytes, suggesting the virtual replacement of the native lymphohematopoietic cell components by those of MHA origin. Susceptibility to epidermolysis induced by MHA–anti-CB cells was demonstrable again 8–12 wk after reconstitution, apparently signifying the return to the circulation of the host's own, i.e. (CB $\times$ MHA)$F_1$, lymphohematopoietic cells bearing CB transplantation antigens. This indirect evidence suggests that these animals were not in fact stable chimeras. The percentage contribution of donor cells apparently increased until the 6th wk after reconstitution, when few or no host cells were detectable. Thereafter, and varying from host to host, the circulating donor cell pool gradually receded in size, obviously at the appearance of the reemergent host cells.

These observations add weight to the proposition that the only source of transplantation antigen that is both necessary and important to the production of overt homologous disease as evidence by epidermolysis in hamsters is leukocytes of peripheral blood,—cells which apparently are constantly leaving the blood stream and traversing the interstitium of the host integument and other tissues. However, this does not imply that all of the transplantation antigenic activity of the skin of the hybrid chimeras is attributable to passenger cells. Fig. 2 shows the median survival times of chimeric-$F_1$ skin grafts on normal MHA recipients. Irrespective of the time of removal of the grafts from their donors after the latter had been rendered chimeric, the skin grafts were ultimately rejected, indicating the unequivocal presence of effective CB antigens. However, beyond the 4th wk after reconstitution, the survival times of these grafts were significantly prolonged as compared to those removed from chimeras of shorter duration or from normal (CB $\times$ MHA)$F_1$ donors. This is interpreted

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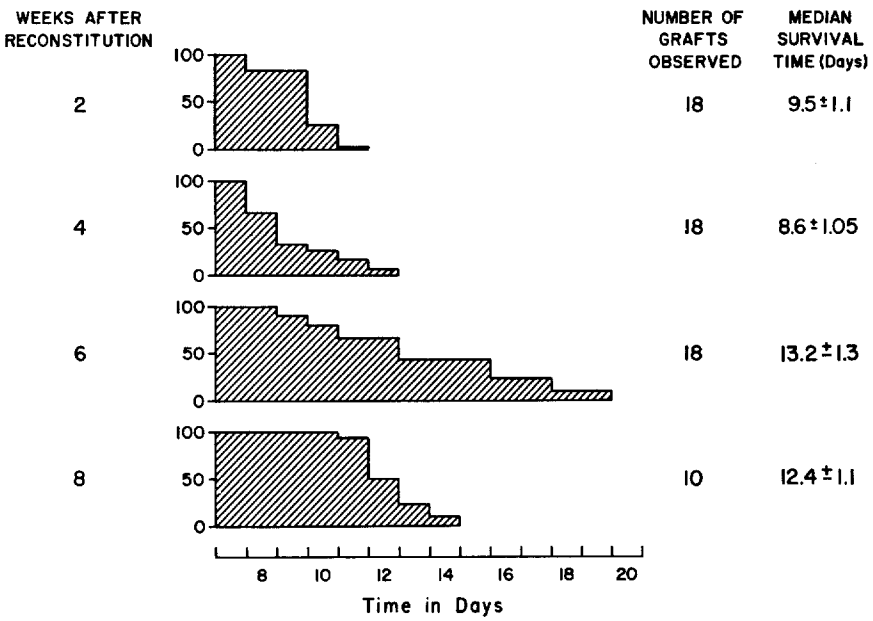


FIG. 2. Per cent survival of Chimeric $F_1$ skin grafts on normal MHA recipients.

as a crude indication of the extent to which passenger leukocytes contribute to the antigenic stimulus of a solid tissue homograft such as skin.

## DISCUSSION

It is well recognized that transplantation antigens are represented on practically all tissues of the body (6). In the early investigations of transplantation or homologous diseases, it was assumed that, because of the ubiquity of these antigens, all host tissues must be more or less at the same risk with regard to the immunologically based destructive process. However, more than a decade of experience with experimentally and clinically induced homologous disease syndromes in different species has demonstrated a great disparity between the ravages of the disease in certain tissues and the apparently trivial involvement of other tissues. Without question, disruption, destruction and atrophy of host lymphatic tissues, including lymph nodes, spleen and thymus, routinely and uniformly form the keystone of the pathologic picture of homologous disease (7–9). In many instances, functional and histological evidences of relative or absolute bone marrow destruction have also been described (10). Although the skin and its appendages are usually affected, the manner and extent of involvement vary such that the lesions may be chronic and mild as in adult mice and

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rats (11), or devastatingly severe as in hamsters. Lesions in the gastrointestinal tract are common in mice and primates, whereas hepatic dysfunction is a prominent feature of graft–versus–host disease in many species (12). By contrast, there is surprisingly little evidence that muscle, bone, gonads, endocrine, renal, or nervous tissues are involved in the destructive process. This discrepancy between the expectations and the observations in GVH disease has focused attention on the need to identify with precision the primary target tissue as a prelude to understanding the pathogeneses of the various manifestations of the disease.

With this in mind, the present demonstration that, despite the extent and severity of the epidermolysis, skin does not serve as a primary immunologic target in acute homologous disease in hamsters seems significant. In the experiments described, the presence of transplantation antigens on the surfaces of epidermal cells proved to be quite superfluous to either the triggering or the sustenance of the immunologic reaction going on within these hosts. When MHA hamsters rendered chimeric with respect to (CB × MHA)$F_1$ leukocytes were injected intracutaneously with lymphoid cells from isologous MHA donors previously immunized to CB transplantation antigens, their integument exhibited severe epidermolysis. This finding indicated that cells of lymphohematopoietic origin were capable in their own right of providing sufficient antigenic stimulus and target to incite a systemic graft–versus–host reaction. Even more impressive was the crucial observation that $F_1$ hybrid hosts whose own circulating pool of leukocytes had virtually been replaced by cells of MHA genetic origin were completely impervious to intracutaneous challenge with suspensions of MHA–anti-CB lymph node cells. Under these circumstances, it was clear that the only relevant source of CB transplantation antigen in the elicitation of epidermolysis and systemic GVH disease was afforded by leukocytic cells. Thus, if leukocytic cells bearing appropriate transplantation antigens are absent from a challenged host animal, neither the antigens present on its own epidermal cells nor on the cells of any other somatic tissue are adequate to induce sufficient GVH reactivity to express itself overtly.

Having identified these peripheral blood leukocytes on circumstantial grounds as the suspected primary targets (at least with respect to epidermolysis), the problem remains of explaining by what means the interaction between these cells and donor attacking lymphoid cells effects the dermal–epidermal dissolution. One simple hypothesis makes the reasonable assumption that the particular location wherein this cellular interaction occurs dictates the type of lesion that ensues: in the epidermolytic syndrome donor cells engage "passenger leukocytes" as they traverse the extravascular spaces of the dermis and subdermis. As a consequence of their interaction, nonspecific, pharmacologically active agents are released—agents with vasoactive, chemotactic, cytotoxic and mitogenic properties—that produce the inflammation and destroy the skin as an "innocent bystander." Experimental evidence in support of the important role

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played by "passenger cells" is available from a variety of sources demonstrating: (*a*) their functional presence as a source of antigen in skin homografts (13); (*b*) their decisive role as contributors of antigen in local graft-versus-host reactions in the skin (14) and in the kidney (15); (*c*) their importance in the genesis of splenomegaly and pock formation on the chorioallantoic membrane of chick embryos (8). Similarly, the notion that immunologic reactions can bring about the destruction of "innocent bystander" cells has also gained firm support from the results of ingeniously designed in vitro experiments (16), as well as suggestive evidence obtained from experiments in intact animals (17–19).

Despite the appeal that the wedding of these two hypotheses affords, it is only fair to point out that none of the evidence presented here precludes the possibility that the donor cell–host leukocyte interaction may actually take place at some more central location—perhaps within the nodes and spleen, rather than in the skin. In that case, as a consequence of this centrally based cellular interaction, it is suggested that there would be released into the general circulation the postulated chemical mediators whose systemic effects would then depend upon the differential susceptibilities of various somatic tissues to their actions. If, on the basis of further experimental evidence, either of these hypotheses proves to be valid, a pathogenetic mechanism will have been provided that would account for the majority of lesions in GVH disease occurring on an immunologically nonspecific basis.

Could it be that cells of lymphohematopoietic origin offer the only important immunologic target to the attacking donor lymphocytes? If so, how can this peculiar vulnerability be accounted for? Clearly, several distinctive cell types are produced from lymphohematopoietic progenitors, and the possibility must be considered that not all of them are at risk of GVH attack. Lafferty and Jones (8), using the chorioallantoic membrane in an allogeneic system, have shown that the production of pocks and the development of splenomegaly depend exclusively upon the interaction of donor lymphoid cells with host *lymphoid* cells (of "reticular" origin). In addition, Heim (20) has recently reported that in adrenalectomized $F_1$ hybrid mice, the only pathologic lesions produced by inoculated parental strain lymphoid cells are found in the so-called thymic dependent regions of the lymph nodes and spleen. The thymus itself, as well as the rest of the node tissue and gut-associated lymphoid structures in these animals, are preserved, suggesting that when, as is usual, these tissues are altered in GVH disease, a second order causative mechanism is operative through the nonspecific agency of the adrenal cortical hormones. If these exciting observations are corroborated by others, the identity of the immunologic target in GVH disease will have been further localized to those anatomic areas containing thymus-dependent lymphocytes. It is then of more than passing interest that the immunologically competent, attacking cells in the donor inoculum are believed also to be thymus-dependent (21). Immunogenetic considerations aside,

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one might expect these donor lymphoid cells, following their injection into an homologous host, to resume their unique and circuitous physiologic pathway, as though in "home territory." Opportunities for immunologic collision of these cells with their counterparts in the host would be found at the major touchstones along their common route—the thymus-dependent regions of the lymph nodes and spleen. It is worth considering the possibility that, once the initial immunological response has thus been joined, the lesions that are found in the skin, in the gut, and in other somatic tissues bear no direct immunologic relationship to the inciting cause; these tissues are destroyed nonspecifically by association or by virtue of contiguity.

## SUMMARY

The epidermolytic syndrome that can be obtained at will in $F_1$ hybrid hamsters by the cutaneous inoculation of adequate doses of parental strain lymphoid cells has been investigated to determine whether the cutaneous lesions are due to an autoimmune process arising from the severe, initial GVH reactions in the skin. It was amply demonstrated that inoculation of donor cells into the skin was of crucial importance to the development of epidermolysis. Parental strain lymphoid cells in similar doses delivered by any other route into normal $F_1$ hybrids failed absolutely to incite the acute syndrome. If "immune lymphocyte transfer" reactions incited by donor cells in the host's skin were surgically removed at timed intervals after inoculation, only complete excision within 24 hr prevented the appearance of epidermolysis in $F_1$ hybrid hosts, indicating that inoculated donor cells must remain within the confines of the skin for approximately 24 hr in order to evoke the disease, persistence for longer periods of time being unnecessary for the subsequent course of the disease. However, reconstitution experiments involving the intramuscular inoculation of suspensions containing mixtures of donor cells and host lymphoid cells, in the presence or absence of epidermal cells, unequivocally indicated that *no* intimate exposure of lymphoid cells to putative skin-specific antigens was essential. Similarly, the elicitation of generalized epidermolysis in $F_1$ hybrids irradiated with 300 r and then inoculated *intravenously* with donor cells casts further doubt on the pathogenic importance of the skin as a source of tissue-specific antigen.

The results of subsequent experiments indicated that host leukocytes, rather than parenchymal cells of the dermis or the epidermis, were important contributors of the transplantation antigenic stimulus. Moreover, a series of experiments, using (CB $\times$ MHA)$F_1$ hybrid hosts that had been lethally irradiated and reconstituted with bone marrow cells from ALS-treated MHA donors, indicated that from 6 to 10 wk after reconstitution—when direct and immune lymphocyte transfer reactions showed a virtual absence of native $F_1$ leukocytes from the circulation—donor cells obtained from specifically sensitized MHA donors were completely ineffective in inducing epidermolysis, while equivalent

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lymphoid cell inocula derived from CB donors evoked the cutaneous disease irrespective of the time elapsed since reconstitution.

To explain these findings it is postulated that in hamsters, the primary targets in graft–versus–host disease incited by the intracutaneous inoculation of donor cells are leukocytes originating in bone marrow or lymph node, or both.

We wish to thank Mr. Steven Tay and Mr. Alan Schneider for gathering data from some of these experiments. Mr. George Sawchuck provided many of the experimental animals and also assisted with some of the work. We are especially appreciative of the excellent and constant technical assistance of Mrs. Joan Streilein without whom this work could not have been accomplished. Dr. Charles Shaffer kindly provided the anti-lymphocyte serum used in these experiments.

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