

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

I. THE EPIDERMOLYTIC SYNDROME: DESCRIPTION AND STUDIES  
ON ITS PROCUREMENT\*

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Syrian hamsters, like other mammals, are capable of responding to the challenge of a homograft with an immunologic attack that swiftly destroys the grafted tissue. In this species the evidence is particularly compelling that homograft rejection is brought about by the action of specifically sensitized lymphoid cells, since it has never been convincingly demonstrated that hamsters can make circulating antibodies to the transplantation isoantigens segregating in available stocks (1). Moreover, hamsters have proven to be particularly useful for the study of transplantation immunity as it is expressed in delayed cutaneous hypersensitivity reactions (2, 3).

During a series of experiments in which normal and immune lymphocyte transfer reactions were being analyzed in hamsters, it was observed that F<sub>1</sub> hybrid subjects that had received intracutaneous inoculations of parental strain lymphoid cells came down with an acute severe cutaneous inflammatory disease. Of particular interest were: (a) the fulminant nature of this disease, (b) the facility and consistency with which it could be procured, and (c) the peculiar nature of the skin lesions, *vis.* the generalized dissolution of the union of epidermis to dermis. The purpose of this communication is to describe in detail this rather unique manifestation of transplantation or homologous disease as it occurs in hamsters, and to present some information relating to its pathogenesis and etiology.

*Materials and Methods*

The hamsters used in these studies were from domestically maintained sublines of the MHA, CB, and LSH isogenic strains. F<sub>1</sub> hybrids were obtained from appropriate matings. Adult animals between 2 and 4 months of age, and weighing between 80 and 120 g, were

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employed. The median survival times of skin homografts exchanged between these strains are listed in Table I (4). This evidence suggests that the CB strain differs from both the MHA and LSH strains by strong histocompatibility factors, while the MHA and LSH strains differ from each other by relatively weak factors.

Skin grafting was carried out according to our standard procedure (5).

Cell suspensions from lymph node, spleen, and thymus, were prepared and dispensed in Hanks' solution by the method of Billingham and Silvers (6).

Intracutaneous inoculations of cell suspensions were made in aliquots of 0.1 ml via No. 28 gauge hypodermic needles into the close-clipped dorsal skin of hamsters that had been anesthetized with chloral hydrate (7). Multiple injections were usually necessary to deliver the required cell dose per recipient.

Specific sensitization of donors of putative "attacking" lymphoid cells was effected by grafting normal animals of one strain with a single skin graft from a homologous strain. Upon rejection of this graft, the hosts were regrafted bilaterally with skin from the original donor strain to boost their sensitivity. When these "second set" grafts were rejected (usually after 8 days), the hosts were killed and cellular suspensions were prepared from their lymph nodes.

Epidermal cell suspensions were prepared by trypsinization of surgically excised hamster cheek pouches according to a method described elsewhere (2). Approximately 15–20 million epidermal cells were obtained from each cheek pouch.

Irradiation was delivered from a 200 kv keleket X-ray source, through a 0.5 mm aluminum filter, at a rate of approximately 55 rads/min and at a distance of 60 cm. During irradiation, the animals were slowly rotated through the X-ray field to ensure an even dosage. The delivered dose was continually monitored by a Victoreen Radacon.

Histologic sections were prepared from surgically excised skin, fixed in Bouin's solution, and stained with hematoxylin and eosin.

The clinical course of each putative victim of homologous disease, after inoculation with lymphoid cells, was followed by daily recording of its body weight and perusal for signs of cutaneous involvement, especially epidermolysis. The criterion by which an animal was judged as suffering from graft-versus-host disease was the unequivocal demonstration of epidermolysis (i.e. a positive Nikolsky sign) in skin where no intracutaneous inoculations had been placed.

#### EXPERIMENTS AND OBSERVATIONS

Homologous or transplantation diseases occur when immunologically competent cells are inoculated into a recipient which, although unable to reject the homologous cellular "graft," expresses transplantation isoantigens determined by a major histocompatibility locus that incite the grafted cells to an immunological response (8, 9). Graft-versus-host (GVH) reactions are obtained experimentally in their simplest form when genetically tolerant  $F_1$  hybrid hosts are inoculated with immunologically competent cells derived from donors of either parental strain. In the experiments that follow, the inoculation of parental strain lymphoid cells into the skins of their  $F_1$  hybrids has been the principal means of procuring homologous disease.

*Clinical Course of Homologous Disease in  $F_1$  Hybrid Hamsters.*—Fifty adult (CB  $\times$  MHA) $F_1$  hybrid animals were inoculated intracutaneously with  $200 \times 10^6$  lymph node cells obtained from MHA donors, previously sensitized against CB tissue antigens. The inocula were subdivided into aliquots of 0.1 cc contain-

ing  $20 \times 10^6$  cells. At each cutaneous inoculation site a typical immune lymphocyte transfer (ILT) reaction developed.

During the first 72 hr, these inflammatory reactions followed a time course identical with that described previously (2). However, by 96 hr and thereafter, the lesions gave no evidence of regression and scar formation, but remained edematous and acquired a sallow devitalized appearance.

At the same time, the entire integument of the affected hamsters became erythematous, the involvement of snout and paws being particularly obvious. By the 7th day after inoculation it was possible, by very light scraping with a fingernail, to remove the epidermis overlying the dermal inflammatory lesions; from any site on the body, large tufts of fur could be pulled out with ease (Fig. 1).

These observations were but the harbingers of the impressive generalized cutaneous events to follow between the 7th and 10th day postinoculation. At this time, large sheets of the intact epidermis could easily be stripped away from affected animals exposing the moist dermal surface. This type of massive epidermal separability to which the dermatologic eponym—positive Nikolsky sign—has been applied, extended to the extremities of the host's integument, including the ears and the paws. Not unexpectedly, affected animals were extremely irritable; they could be brought to a frenzy of senseless activity if their cages were accidentally tapped.

The animals' body weights remained relatively stable through the initial 9–10 day period but began to fall thereafter. As crusting of the mucous membranes of the mouth, nares, and eyes became prominent on days 11–12, weight loss became precipitous. Almost without exception, the rapid downhill course exhibited by each animal culminated in death between 15 and 21 days after inoculation, by which time it had lost upwards of 40% of its original body weight (Fig. 1).

*Histologic Findings in Skin from Affected Animals.*—Histologic examination of samples of skin obtained from animals injected with sensitized parental strain cells 7 days previously revealed that, in areas in which epidermolysis had not yet become apparent and which were remote from inoculation sites, there was infiltration of the dermis by moderate numbers of mononuclear cells as well as by infrequent polymorphonuclear leukocytes and mast cells. The cells of the basal layer of the superficial epidermis were vacuolated but intact. The dermis was edematous; venules were dilated and engorged with erythrocytes, and there was some perivascular cuffing with round cells.

By the 10th day, sections of skin in which epidermolysis was extant revealed dissolution of the dermal-epidermal junction, apparently at the expense of the basal epidermal cells. Many of these cells were disrupted, the remains of the superficial epidermis lying free. No change in the dermal cellular infiltrate was

noted at this time and, more specifically, there was no accumulation of round cells in the superficial dermis (Fig. 2).

*Viability Studies on Skin from Affected Animals.*—On the basis of both the gross and histologic appearance of these cutaneous lesions, it appeared that the underlying pathogenic process was directed at putative targets at or near the dermal-epidermal junction. It seemed important, therefore, to determine by grafting experiments whether the exfoliated sheets of epidermis and/or the denuded, inflamed dermis beneath were themselves viable or contained viable elements. Accordingly, sheets of stripped epidermis or grafts of raw dermis from

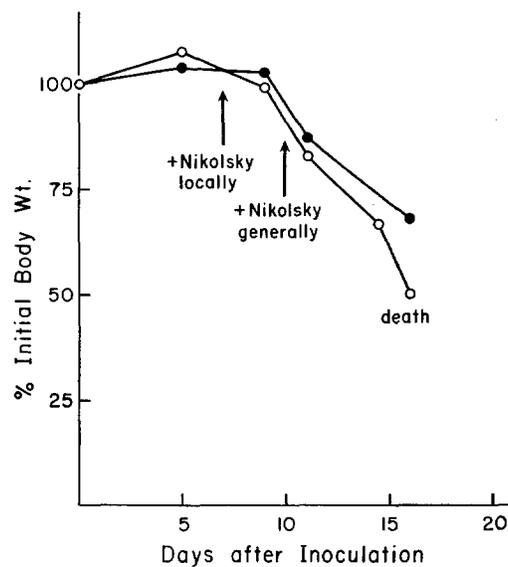


FIG. 1. Typical clinical course of two (CB  $\times$  MHA) $F_1$  hybrid hamsters that had received intracutaneous inoculations of  $200 \times 10^6$  MHA-anti-CB lymph node cells.

(CB  $\times$  MHA) $F_1$  animals exhibiting severe epidermolysis as the result of the intracutaneous inoculation of  $200 \times 10^6$  MHA-anti-CB lymph node cells 10 days previously, were transplanted to full-thickness beds prepared on the lateral thoracic wall of normal (CB  $\times$  MHA) $F_1$  hybrid hamsters. When inspected on the 8th postoperative day, both types of grafts had healed in well. The initially raw dermal grafts had become partially resurfaced, apparently as a consequence of the migratory activity of residual follicular epithelium. These grafts were completely resurfaced by 10 days and subsequently regenerated sparse crops of fur. The wounds which received the stripped epidermal sheets displayed varying degrees of resurfacing by healthy-looking hyperplastic epithelium, signifying the survival of at least some malpighian cells in the grafts. These findings indi-

cate that, if the target of the immunologic attack was the basal epidermal layer, some cells were spared this destruction and that the essential structure of the dermis was not irreversibly damaged.

*Immunogenetic Requirements for Production of the Disease.*—Using the three inbred hamster strains and their F<sub>1</sub> hybrids, we carried out experiments to determine under which immunogenetic conditions the epidermolytic disease could be incited. In each donor/host combination tested, the F<sub>1</sub> hybrids received a standard inoculum of  $200 \times 10^6$  lymphoid cells derived from specifically sensi-

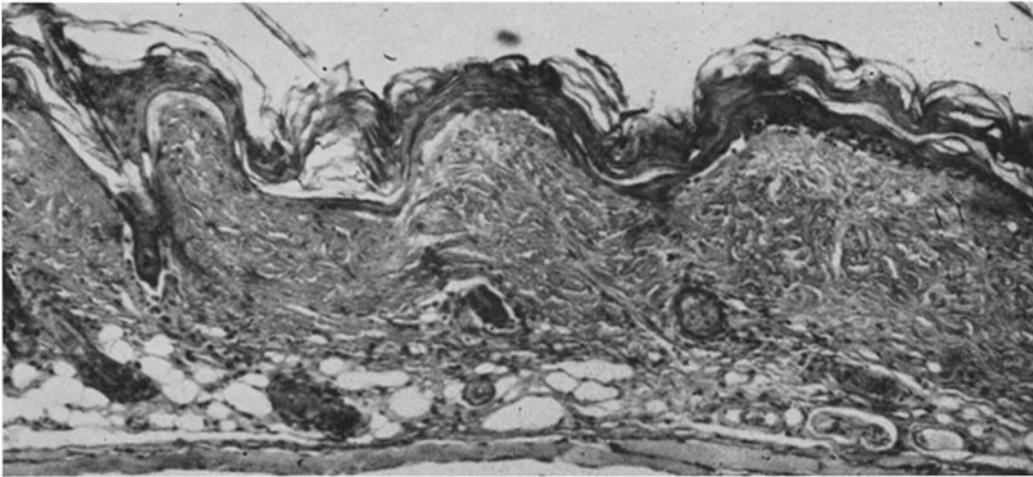


FIG. 2. Section through trunk skin of a (CB  $\times$  MHA)F<sub>1</sub> hybrid hamster suffering from acute toxic epidermolytic disease as a consequence of intradermal inoculation 10 days previously with  $200 \times 10^6$  lymph node cells from MHA strain hamsters sensitized against CB strain tissue antigens. Note the hyperkeratotic superficial epidermis which is separating from the underlying dermis, and the lytic disintegration of many of the basal layer cells.  $\times 10$ .

tized donors of one parental strain. The findings summarized in Table I indicate that, with both the (CB  $\times$  LSH)F<sub>1</sub> and (CB  $\times$  MHA)F<sub>1</sub> hosts, lymphoid cells from *either* parental strain were equally capable of inducing acute epidermal necrolysis. However, parental strain cells injected into (MHA  $\times$  LSH)F<sub>1</sub> hamsters were completely innocuous, even when the inoculum was increased to  $400 \times 10^6$  cells/recipient. This evidence supports the idea that CB animals differ from LSH and MHA hamsters by a "major" histocompatibility factor and is in keeping with observations regarding the potential for graft-versus-host disease among strains of other species differing widely with respect to histoincompatibility (9, 10).

Utilization of genetically tolerant F<sub>1</sub> hybrids was not obligatory to the pro-

duction of the epidermolytic syndrome. The inoculation of  $200 \times 10^6$  MHA-anti-CB lymphoid cells into adult CB hosts and similar numbers of CB-anti-MHA cells into MHA hosts also regularly produced the acute skin syndrome. However, unlike the  $F_1$  hybrids, these animals usually made a complete recovery attributable, no doubt, to their development of sensitivity directed against the attacking cells. The fact that recovered animals gave intense direct reactions (11) when challenged intracutaneously with donor strain cells, and rejected skin grafts from this strain in an immune manner, adds evidence to this interpretation. As expected, specifically sensitized MHA lymphoid cells were in-

TABLE I  
*Immunogenetic Requirements for Elicitation of Homologous Disease (Epidermolysis) in Adult Hamsters of Various Isogenic Strains*

Lymphoid cell donor strain	Recipients*	Number tested	MST† of recipient skin on donor strain	No. of recipients with epidermolysis		Died of disease	
			days	%	%	%	
MHA	(CB × MHA) $F_1$	50	11.0 ± 0.5	50	(100)	49	(98)
CB	(CB × MHA) $F_1$	10	13.0 ± 1.0	10	(100)	10	(100)
MHA	CB	15	11.2 ± 0.5	15	(100)	0	(0)
CB	MHA	8	11.9 ± 0.7	8	(100)	1	(12)
LSH	(CB × LSH) $F_1$	11	11.2 ± 0.6	11	(100)	11	(100)
CB	(CB × LSH) $F_1$	8	13.5 ± 1.1	8	(100)	8	(100)
MHA	(MHA × LSH) $F_1$	15	28.0 (24.4–38.36)	0		0	
LSH	(MHA × LSH) $F_1$	14	16.2 (14.1–18.64)	0		0	

\* Each recipient was inoculated with  $200 \times 10^6$  lymphoid cells from specifically sensitized members of the donor strain.

† MST, median survival time.

capable of producing any overt signs of graft-versus-host disease in adult LSH hamsters.

*Identification of Inciting Cell Type.*—Under the reasonable assumption that the GVH reactivity was in some way responsible for the cutaneous lesions described above, we prepared monodisperse suspensions of viable cells from the following tissues and organs: regional lymph nodes, nondraining lymph nodes, spleen, peripheral blood (leukocytes), thymus, bone marrow, peritoneal exudates (macrophages), and cheek pouch (epidermis). Their relative capacities to evoke the acute disease were delineated. MHA hamsters that were sensitized to CB tissue antigens were the donors of all cell types employed.

Table II summarizes the experimental results. The findings show very clearly that only those tissues known to contain high proportions of immunologically competent lymphocytes are capable of producing the acute disease. These findings are compatible with the hypothesis that the syndrome under investigation is, in fact, the end result of a graft-versus-host reaction.

*Influence of Cell Dosage on Procurement of Epidermolysis.*—If the epidermal necrosis displayed by affected  $F_1$  hamsters is the result of GVH reactivity on the part of lymphoid cells, then the capacity to produce the syndrome ought to be dependent upon two factors: (1) the immunologic status of the donor of the attacking cells, whether normal or specifically sensitized, and (2) the total number of donor cells inoculated. For evaluation of the importance of these parameters, a series of tests were carried out in which the dosage of donor cells

TABLE II  
*Capacity of Cellular Inocula Derived from Various MHA\* Tissues to Produce Epidermolysis in  $(CB \times MHA)F_1$  Hosts*

Tissue source of donor cells	No. of cells inoculated intracutaneously into each $F_1$ host ( $\times 10^6$ )	No. hosts challenged	No. of hosts exhibiting epidermolysis	%
Lymph nodes				
All	50	13	13	(100)
Draining	40	6	6	(100)
Nondraining	40	6	0	(0)
Spleen	50	11	11	(100)
Thymus	200	8	0	(0)
Buffy coat (from peripheral blood)	50–90	6	4	(67)
Bone marrow	200	8	0	(0)
Peritoneal exudate	125	4	0	(0)
Epidermis (from cheek pouch)	50	6	0	(0)

\* All MHA donors had been specifically sensitized to CB transplantation antigens as described in Materials and Methods.

in the cutaneous inocula was gradually reduced from the standard of  $200 \times 10^6$  per recipient, until a threshold was reached below which no overt sign of epidermolysis occurred. The cell donors were either normal MHA animals or MHA hamsters that had been presensitized to CB transplantation isoantigens.

The findings summarized in Table III show that over the broad range of  $50$ – $200 \times 10^6$  MHA-anti-CB node cells, 100% of  $F_1$  recipients developed epidermolysis and upwards of 80% died. By contrast, lymphoid cell inocula from unsensitized MHA donors were less effective— $50 \times 10^6$  cells were nonlethal and incited epidermolysis in only 50% of tested animals. It is clear that at threshold

or slightly subthreshold doses of attacking cells, recipients often developed mild to moderate epidermolysis, and most recovered from this disorder.

Although an inoculum of  $25 \times 10^6$  specifically sensitized MHA cells was capable of inciting the nonlethal form of the disease, none of the  $F_1$  hybrid hamsters receiving  $25 \times 10^6$  normal MHA lymphoid cells came down with the cutaneous syndrome.

Several investigators have demonstrated that prior exposure of an  $F_1$  hybrid host to sublethal whole body X-irradiation significantly heightens the animal's susceptibility to homologous disease (8). In a series of studies bearing on this

TABLE III  
*Influence of Immunologic Status and Dosage of MHA Lymph Node Cells on their Capacity to Evoke Epidermolysis following Intracutaneous Inoculation in (CB  $\times$  MHA) $F_1$  Hamsters*

Immunologic status of donor	No. of cells inoculated ( $\times 10^6$ )	No. of $F_1$ hosts tested	No. of which developed epidermolysis		No. of which succumbed to acute diseases	
				%		%
Specifically sensitized	200	50	50	(100)	49	(98)
	100	10	10	(100)	8	(80)
	50	13	13	(100)	11	(86)
	25	8	4	(50)	0	(0)
	12.5	6	0	(0)	0	(0)
Unsensitized	200	10	10	(100)	10	(100)
	100	10	8	(80)	7	(70)
	50	10	5	(50)	0	(0)
	25	6	0	(0)	0	(0)
Specifically sensitized. Hosts pretreated with 300 R.	50	10	10	(100)	10	(100)
	25	8	8	(100)	8	(100)
	12.5	8	8	(100)	8	(100)
	6.25	8	8	(100)	6	(75)
	3.125	8	5	(62)	1	(12)

question, (CB  $\times$  MHA) $F_1$  hybrid hamsters were exposed to 300 R whole body irradiation. Twenty-four hr later, various panels were challenged intracutaneously with decreasing numbers of either MHA-anti-CB or normal MHA lymphoid cells. The results (Table III) indicate that preirradiation exerts a considerable reduction (about 8 fold) in the dosage of cells required to cause the overt disease in hamsters. That the astonishingly low level of  $3 \times 10^6$  specifically sensitized cells was capable of producing epidermolysis in sublethally irradiated  $F_1$ 's was unexpected, but the dosage reduction was consistent with the experience of others. Taken at face value, these data suggest that cell suspensions derived from specifically sensitized donors contain approximately two to four

times the number of antigen-reactive cells as do their unsensitized, normal counterparts.

*Adoptive Transfer of Epidermolysis from Affected to Normal F<sub>1</sub> Hybrid Hosts.*—The ability to transfer transplantation disease from affected to normal hosts is a natural accompaniment and requirement of the GVH hypothesis. Other workers have reported that homologous disease can be transferred, albeit with some difficulty, by means of lymphoid cells but not by means of serum from affected donors in mice and rats. Transfer experiments were carried out in appropriate F<sub>1</sub> hybrid hamsters and the results are presented in Table IV. Using a ratio of one affected donor to one normal recipient, we could regularly transfer the disease by using cells harvested from the hypertrophied nodes of hybrid donors that had been inoculated intracutaneously with  $200 \times 10^6$  MHA-anti-

TABLE IV  
*Adoptive Transfer of Epidermolysis from Primary (CB × MHA)F<sub>1</sub> Animals Previously Inoculated with  $200 \times 10^6$  MHA-anti-CB Lymph Node Cells into Secondary Hosts\**

Genetic constitution of host	Number inoculated	Number which developed epidermolysis	Number which succumbed
(CB × MHA)F <sub>1</sub>	13	13	7
MHA	8	0	0
MHA pretreated with 300 R	5	0	0
CB	8	2	0
CB pretreated with 300 R	7	2	2

\* Each secondary host received intracutaneously all the lymph node cells that could be harvested from one primary recipient, 7 days after its original inoculation with sensitized cells.

CB lymphoid cells 7 days beforehand. It should be pointed out that, whereas approximately  $100\text{--}125 \times 10^6$  cells represent an average yield from the accessible lymph nodes of a normal adult F<sub>1</sub> hamster, the nodes from F<sub>1</sub> hybrids affected by GVH disease yielded between 400 and  $600 \times 10^6$  cells/animal. Several attempts were made to transfer the acute cutaneous disease to normal F<sub>1</sub> hybrids with serum harvested from affected F<sub>1</sub> donors, but even when 10 donor equivalents were administered to one recipient (a total of 25 cc of serum) whether in single shot fashion or in subdoses over a 7-day period, no evidence of GVH disease appeared.

In a variation on these experiments, panels of MHA or CB hosts were challenged intracutaneously with lymphoid cells derived from affected (CB × MHA)F<sub>1</sub> hybrids initially injected with  $200 \times 10^6$  MHA cells. In no instance did any MHA recipients develop epidermolysis, whereas two of eight normal CB hosts, and two of five irradiated CB hosts did exhibit epidermal necrolysis (Table IV).

Experiments were then carried out to determine how soon, after the inoculation of primary  $F_1$  hybrids with presensitized MHA lymphoid cells, adoptive transfer of the disease could be achieved.  $(CB \times MHA)F_1$  hybrids were each inoculated intracutaneously with  $200 \times 10^6$  MHA-anti-CB lymphoid cells; beginning 24 hr later, panels of these animals were killed at daily intervals through 7 days, and lymph node cell suspensions prepared therefrom. These cells were transferred into the skins of normal  $(CB \times MHA)F_1$  secondary hosts, maintaining a ratio of one donor lymphoid cell equivalent per recipient. As indicated in Table V, although successful passage of the disease could be achieved by the 4th day after MHA challenge and thereafter, no sign of epidermolysis or any other manifestation of GVH disease developed in  $F_1$  hybrids receiving lymph node cell suspensions from donors inoculated less than 4 days previously.

TABLE V

*Ability to Transfer Epidermolysis Adoptively in Relation to Time Elapsed since Inoculation of MHA-anti-CB Lymph Node Cells Intracutaneously into Primary  $(CB \times MHA)F_1$  Hosts*

Character of transferred cells	Days after primary inoculation	Appearance of epidermolysis in secondary $(CB \times MHA)F_1$ hosts					
		1	2	3	4	5	7
Lymph node cell suspension	Number of secondary hosts with epidermolysis	0	0	0	6	9	10
	Number of secondary hosts challenged	4	4	10	10	10	10
Lymph node fragments	Number of secondary hosts with epidermolysis	0	2	9	4		
	Number of secondary hosts challenged	4	4	10	4		

This apparent requirement for a minimum "incubation" time of 4 days was successfully circumvented in experiments in which fine lymph node fragments, rather than monodispersed cell suspensions, were transferred intraperitoneally to normal  $F_1$  hosts. Animals that received one donor equivalent of lymph node fragments harvested from  $F_1$  hybrid donors as early as 2 days after receipt of presensitized MHA lymph node cells developed generalized epidermolysis as well as other signs of homologous disease. This discrepancy between the reduced efficacy of suspended cells, as compared with minced lymph node tissues, may reflect heightened fragility of the essential cell components in the nodes at the second and third day. Alternatively, it may have as its basis certain requirements for the maintenance of intact anatomical associations between cells within the node which the suspending process would of necessity destroy. The experimental findings do not permit a choice for either of these options.

The final set of cell transfer experiments was designed to determine through how many serial passages the disease could be successfully taken. Early in the history of GVH syndromes, Simonsen had reported as many as six successful serial passages using outbred chicks (12). However, since then most investigators using isogenic rodent strains, as well as inbred chicken strains, have only been able to achieve one or two passages before the cell suspensions lost their capacity to incite the disease (13, 14).

Attempts at serial passage of the disease in hamsters beyond the tertiary host by means of cells were unsuccessful. In these experiments, each of eight (CB  $\times$  MHA) $F_1$  hamsters received  $200 \times 10^6$  MHA-anti-CB lymphoid cells intracutaneously. 7 days later, their lymph node cells were harvested and pooled, and divided amongst eight normal (CB  $\times$  MHA) $F_1$  secondary hosts. After another 7 day wait, all the lymph nodes were harvested from these secondary hosts, pooled, and delivered intracutaneously to another panel of similar animals constituting the tertiary hosts. Only two of these animals exhibited minimal signs of epidermolysis. When lymph node cell suspensions were prepared from these animals and inoculated into yet another panel of normal (CB  $\times$  MHA) $F_1$  hybrids, no sign of epidermolysis appeared. In a similarly designed experiment, eight (CB  $\times$  MHA) $F_1$  hybrids were irradiated with 300 R prior to challenge with lymph node cells derived from eight tertiary hosts. No epidermolysis developed in these animals.

As a final effort at achieving more serial passages of the disease, a telescoping design was adopted in which, at each passage, the number of lymphoid cell donors exceeded the number of recipients by a factor of two (Fig. 3). In addition, the final recipient received a sublethal dose of whole body X-irradiation prior to attempted transfer.

Three such experiments were carried out (one of which is summarized in diagrammatic form in Fig. 3), despite the overwhelming demands that were made for animals. It is of interest to note the total numbers of lymphoid cells harvested and transferred at each level of passage. At the outset,  $2.4 \times 10^9$  MHA-anti-CB lymph node cells were inoculated into twelve primary  $F_1$  hosts ( $200 \times 10^6$  cells into each). 7 days later,  $4 \times 10^9$  lymphoid cells were harvested from these animals, pooled, and then injected in divided, equal doses into the six secondary hosts. After another 7 day interval, a total of  $1.5 \times 10^9$  cells was obtained for transfer to three tertiary hosts, who after 7 additional days, could yield in aggregate only a paltry  $300 \times 10^6$  lymphoid cells. Despite the fact that at each passage the suspended cells were reconfronted with  $F_1$  antigens at the inoculation sites, this did not appear to offer a sufficient stimulus to their continued multiplication. In fact, one wonders whether the effect of serial transfer was stimulating in any regard, since after three adoptive transfers, an original donor inoculum of  $2.4 \times 10^9$  cells was reduced to only  $300 \times 10^6$  cells for final passage. In all three experiments, only  $\frac{2}{3}$  of the tertiary recipients exhibited mild epidermolysis, while none of the final recipients demonstrated any disease

whatever, despite their prior exposure to 300 R. Considering the previous observations that sublethal irradiation reduced the threshold to epidermolysis from  $50 \times 10^6$  attacking cells to only  $3 \times 10^6$ , it was surprising that the  $300 \times 10^6$  cells obtained from the tertiary hosts apparently lacked even this number of attacking cells.

*Immunogenetic Specificity of Epidermolysis.*—Previous investigations have shown that the cutaneous lesions of GVH syndromes in rats and mice develop only in skin bearing transplantation antigens alien to the attacking cells, while an established skin graft isogamous to the donor attacking cells remains un-

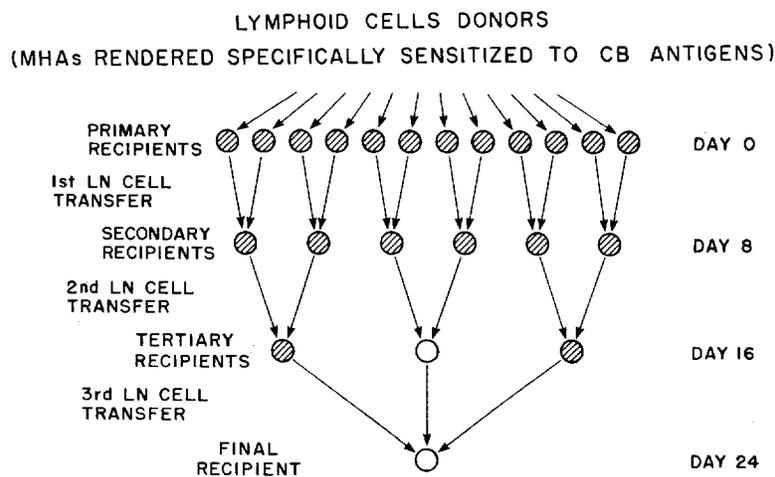


FIG. 3. Experimental protocol and results of attempted serial passage of epidermolysis in  $(CB \times MHA)F_1$  hamsters. LN cells were transferred by intracutaneous inoculation into recipients. Open circle indicates recipients exhibiting no epidermolysis; cross-hatched circle indicates that the recipient exhibited epidermolysis before sacrifice or termination of experiment; the final recipients were exposed to 300 R whole body irradiation 24 hr before transfer of lymph node cells from tertiary recipients.

molested (13, 15). To test the specificity of the epidermolytic process in  $F_1$  hamsters, we grafted 12  $(CB \times MHA)F_1$  hamsters orthotopically with full thickness grafts from both MHA and CB strain donors. These grafts healed in well as anticipated. When they had developed a full crop of appropriately colored fur, their hosts were challenged with MHA-anti-CB lymphoid cells delivered into the  $F_1$ 's own integument. As expected, each of the animals developed the acute cutaneous disease and, as the epidermolysis proceeded through its own skin, it also affected the CB strain homograft. However, in each host at the peak

of the cutaneous involvement, the epidermis could also be peeled from the raw dermis of the *MHA* strain graft, i.e., skin having exactly the same genetic and antigenic constitution as the attacking cells (Table VI).

This quite unexpected result prompted a second experiment in which  $F_1$  hybrids, also bearing large full-thickness *MHA* strain grafts, received the challenge inocula of *MHA*-anti-*CB* lymphoid cells directly and exclusively into the skin of the well-established *MHA* grafts. Intense *ILT* reactions developed at these inoculation sites and inevitably these  $F_1$  hosts shared the same epidermolytic fate as their predecessors (Table VI).

TABLE VI  
*Studies on Immunologic Specificity of Epidermolysis Produced in (CB × MHA) $F_1$  Hosts by Intracutaneous Injection of Lymphoid Cells from MHA Donors Specifically Immunized to CB Antigens*

Recipients	Number challenged with <i>MHA</i> -anti- <i>CB</i> lymph node cells	Site of challenge inocula	Results
( <i>CB</i> × <i>MHA</i> ) $F_1$ hybrids bearing <i>MHA</i> and <i>CB</i> skin homografts	12	$F_1$ hybrid skin	12 developed generalized epidermolysis which also involved <i>CB</i> and <i>MHA</i> grafts. All died.
( <i>CB</i> × <i>MHA</i> ) $F_1$ hybrids bearing <i>MHA</i> and <i>CB</i> skin homografts	8	<i>MHA</i> graft	8 developed generalized epidermolysis and died.
<i>MHAs</i> chimeric with lymph node and bone marrow cells from ( <i>CB</i> × <i>MHA</i> ) $F_1$ donors	15	<i>MHA</i> skin	13 developed generalized epidermolysis, from which 10 died.

To try and elucidate the apparent lack of immunogenetic specificity in the genesis of the cutaneous lesions, a panel of 15 adult *MHA* hamsters received a potentially lethal dose of irradiation (900 R). 24 hr later they were rehabilitated by the intravenous inoculation of suspensions of bone marrow and lymph node cells derived from normal (*CB* × *MHA*) $F_1$  donors. In a preliminary experiment, 10 similarly treated animals survived and developed a high degree of specific immunologic tolerance to *CB* tissue isoantigens as evidenced by their indefinite acceptance of subsequently transplanted grafts of *CB* strain skin. 6-8 wk following reconstitution, chimeric *MHA* hamsters, some of which bore normal-appearing *CB* skin grafts but others of which had received no such graft, were challenged intracutaneously with  $200 \times 10^6$  *MHA*-anti-*CB* lymphoid cells

(Fig. 4). Typical immune lymphocyte transfer reactions ensued at the injection sites, followed by local epidermolysis which proceeded to envelope the *entire* animal. Most of the MHA chimeras succumbed to the acute cutaneous disease.

Since the only source of CB transplantation antigens in these animals was that present on blood-borne cells of bone marrow or lymph node origin, it seemed inconceivable that the epidermolysis could be the result of a direct attack of sensitized cells, seeking out and destroying epidermal cells bearing the appropriate homologous transplantation antigens.

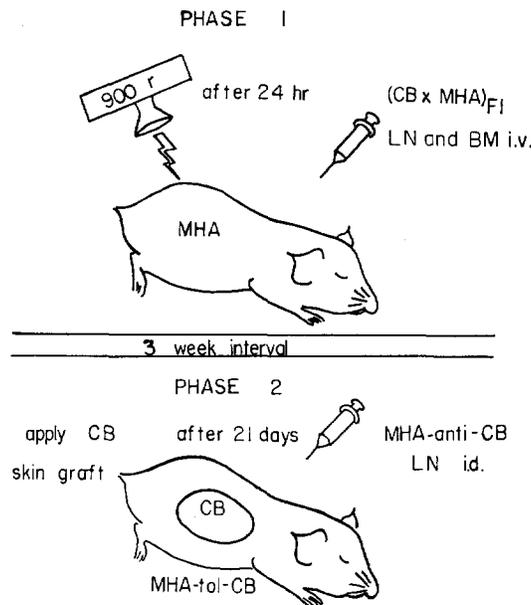


FIG. 4. MHA animals rendered tolerant and chimeric with respect to  $(CB \times MHA)_{F1}$  leukocytes in this manner were employed as hosts in experiments bearing on the immunologic specificity of epidermolysis.

#### DISCUSSION

Since the original, classic descriptions of graft-versus-host disease in experimental animals, a great deal has been learned about the conditions under which these syndromes can be procured and about their manifestations in various species. However, it seems fair to say that there is still very little insight into the mechanisms by which the lesions of homologous disease come about. The studies reported here, and in the reports to follow, have been undertaken in an attempt to shed new light on this subject.

Much about the epidermolytic syndrome described here in hamsters resembles GVH disease in other species: the dependence upon a major histoin-

compatibility antigen difference between donor and host; the pivotal role played by lymphoid cells in its procurement; the superior ability of specifically sensitized as compared to unsensitized lymphoid cells in inciting the disease; and the capacity to passage the disease from affected to normal animals by cells, rather than by serum. Other aspects of the condition, as expressed in hamsters, are unique. The cutaneous lesion—a complete cleavage at the dermal-epidermal junction—has not been described before in a laboratory animal and resembles in many ways the human skin condition described by Lyell, toxic epidermal necrolysis (16). Although there is no reason to implicate a graft-versus-host mechanism as a cause of this human skin disorder, it would be interesting to know whether some patients treated with homologous bone marrow transplants will develop epidermolysis as an expression of a systemic graft-versus-host disease. Whether or not the hamster disease can serve as a model for the human disorder is unclear, but the similarity of the syndromes in terms of rapidity of onset and involvement of the *entire* integument suggests at least that a similar effector mechanism may underly both.

Several points emerge from the data presented that warrant further discussion in the context of transplantation disease in general. First, the cell dosage experiments reawaken old doubts about our understanding of the procurement of graft-versus-host diseases. Reduced to its purest form, the question can simply be stated: If graft-versus-host disease is the direct result of an attack upon defenseless host tissue by immunologically competent donor lymphoid cells, why should a threshold exist for the number of donor cells? Theoretically, one or at most only a very few antigen-sensitive donor cells should be capable of repeated division in response to confrontation with host transplantation antigens, such that the descendants could procure the host's ultimate destruction. Yet, at a minimum,  $25 \times 10^6$  MHA-anti-CB cells are required to produce signs of epidermolysis in susceptible F<sub>1</sub> hamsters, and this requirement can be reduced to  $3 \times 10^6$  cells by exposing the recipient to sublethal irradiation. But this observation in itself gives no clue as to the reason for the existence of an apparent threshold.

Second, the results of the serial transfer experiments reinforce the findings of others that the capacity of a particular inoculum of attacking cells to evoke the disease is dissipated with time (17), such that in the hamster experiments only two passages were possible before the descendants of these cells became ineffective in causing the disease. Moreover, the results of the "telescoping" transfer experiments make the possibility of progressive dilution of original donor cells a rather unlikely cause of this incapacity.

These observations, when viewed in the light of the cell-dosage data, suggest that: (a) the responding cells of the original inoculum either are capable of only a finite number of mitotic divisions or are transformed into a cell type that has lost its capacity to attack and kill target cells, or (b) some of the prog-

eny of each mitotic generation undergo destruction when confronted by antigenically foreign host cells.

With this in mind, the finding that the disease could be transferred 3 days after initial inoculation of donor cells only if the lymph nodal architecture was preserved, takes on added significance. Impressive evidence has now been attained implicating two cell lines in the production of circulating antibody to certain antigens (18). If cellular immunity is comparably designed for some antigenic stimuli, then in the experiments reported here this vulnerability of affected lymph nodes to disruption at two and three days may reflect an analogous instructional and/or recruiting period from which the ultimate effector cells will emerge.

Perhaps the most unexpected finding of all was that the epidermolysis found in affected animals was not the result solely of a direct attack in which MHA lymphoid cells sought out and destroyed epidermal cells bearing alien CB antigens. The experiments employing MHA hosts made chimeric with respect to (CB × MHA) $F_1$  leukocytes clearly demonstrated that one cannot account for the epidermal necrolysis by simply invoking a direct graft-against-host mechanism.

Ramseier and Billingham had previously shown that in order for an immune lymphocyte transfer reaction to develop in hamsters, the only relevant and important source of transplantation antigens was blood-borne cells, passing through the skin at the time of injection (2). Although it was conceivable that this mechanism might produce epidermolysis in  $F_1$  hybrid hamsters, a much more attractive hypothesis was advanced to explain the pathogenesis of this unique cutaneous lesion: As a consequence of the confrontation within the skin of immunologically competent MHA lymphoid cells with CB isoantigens, an inflammatory reaction ensued that resulted in the unmasking of certain epidermal-specific antigens, to which lymphoid cells of donor or host origin could then respond, thereby setting up an autoimmune response as the basis of the epidermolysis. In the report to follow, this hypothesis is put to experimental test.

#### SUMMARY

$F_1$  hybrid hamsters derived from genetically disparate strains develop a severe and often lethal cutaneous disorder when inoculated intracutaneously with immunologically competent lymphoid cells from either parental strain. The disease is characterized clinically by extensive epidermal necrolysis, and histologically by a complete dissolution of the dermal-epidermal junction. The requisites for elicitation of this syndrome were determined to be: (a) the parental strains must differ from each other at a major histocompatibility locus, and (b) the donor inoculum must contain immunologically competent parental strain cells. In addition it was found that specifically sensitized cells surpassed

normal unsensitized ones in their ability to elicit the disease, and that the disease can be transferred adoptively from affected to normal F<sub>1</sub> hosts by means of lymphoid cells. On the basis of these observations, it was concluded that the disease was immunologic in nature, and graft-versus-host in type. However, a series of critical studies failed to demonstrate that the epidermolysis had an immunogenetically specific basis, thus invalidating the provisional assumption that this lesion resulted from a direct immunologic attack upon parenchymal cells of the epidermis and dermis. With the aid of radiation chimeras, it was clearly established that typical epidermolysis could be induced in skin of the same genetic constitution as the attacking donor lymphoid cells. This paradox was taken into account by the possibility that, amid the intense local cutaneous graft-versus-host reactions, "skin-specific" antigenic determinants are bared which incite a quasi autoimmune response that in turn is responsible for the epidermolytic lesions.

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