STUDIES ON NORMAL AND IMMUNE LYMPHOCYTE TRANSFER REACTIONS IN GUINEA PIGS, WITH SPECIAL REFERENCE TO THE CELLULAR CONTRIBUTION OF THE HOST

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(Received for publication 31 August 1972)

After the initial discovery and careful analysis of both normal and immune lymphocyte transfer reactions (NLT and ILT reactions) in guinea pigs (1–4), these cutaneous manifestations of transplantation immunity have been studied in a variety of species including dogs (5), rabbits (6, 7), hamsters (8), rats (9), mice (10), and men (11). They have been employed as a means of tissue matching, elucidation of the mode of action of immunosuppressive agents, and of the modus operandi of homograft and graft-versus-host (GVH) reactions.

All workers are agreed that transfer reactions are initially of the local GVH type though there are good grounds for belief that, in appropriate genetic contexts, host-versus-graft reactivity, i.e. “direct hypersensitivity reactions” (3), may intervene and complicate the situation. Various findings have established that in mice, rats, and hamsters the principal host target cells in these reactions, as in other local as well as in systemic forms of GVH reactivity, are radiosensitive cells of hematologic origin, probably lymphocytes, rather than fixed or constitutive cells of particular nonlymphohematopoietic tissues and organs where these reactions are made to take place (12, 13). For example, a rat’s or a hamster’s capacity to manifest GVH reactivity can be abrogated by a dose of whole body irradiation sufficient to reduce its peripheral leukocyte count to a low level. Furthermore, mice and hamsters rendered tolerant of (and chimeric with respect to) bone marrow and circulating leukocytes from an unrelated donor strain are capable of responding by cutaneous reactions when inoculated intradermally with lymphoid cells from donors of their own genetic constitution.

However, Brent and Medawar (2, 4) found that exposure of guinea pigs to 600 or 1500 R whole body irradiation before lymphocyte transfer, which they maintained delayed or abolished the host’s capacity to counterattack the transferred cells, did not impair their capacity to sustain transfer reactions. This observation appeared to differentiate the guinea pig’s behavior very strikingly from that of hamsters and rats, as did the finding that the tempo of the skin reactions in guinea pigs is gen-

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1 Abbreviations used in this paper: GVH, graft-versus-host; ILT, immune lymphocyte transfer; NLT, normal lymphocyte transfer.
erally quicker than in the other species. This raised the possibility, favored by Brent and Medawar, that in guinea pigs the antigens with which donor lymphocytes engage are already present in the host’s skin, i.e., are associated with its constitutive cells.

The work to be described was undertaken principally to define the host target cell in these reactions and to evaluate the potential of heavily irradiated guinea pigs to develop delayed cutaneous hypersensitivity to alien cellular transplantation antigens.

Materials and Methods

Experimental Subjects.—These were young adult, 300-500 g guinea pigs of both sexes belonging to domestically maintained sublines of the isogenic strain Nos. 2 and 13 and their F1 hybrids.

Suspensions of Viable Cells.—Lymphocytes from peripheral blood, lymph node cells (hereafter “node cells”) from all accessible nodes, and bone marrow cells were prepared and dispensed in Hanks’ solution according to procedures described elsewhere (14, 15).

Antigenic Extract.—This was prepared from pooled node and splenic cell suspensions and dispensed in phosphate-buffered saline (16).

Sensitization of Guinea Pigs.—Sensitization against tissue antigens was accomplished either by means of a primary orthotopic skin homograft or intradermal injection, at multiple sites, of an aggregate dosage of \( 60 \times 10^6 \) homologous node cells followed, 1 wk before use, by a “booster” dose of \( 60 \times 10^6 \) node cells administered both intraperitoneally and intracutaneously. Animals that responded to intradermal challenge with donor antigen by direct reactions (see below) of 3+ or greater intensity were designated as “sensitized” and used as such.

Skin Testing and Scoring.—Standard cell inocula for use as test antigen in direct reactions, or for inciting transfer reactions, comprised \( 20 \times 10^6 \) node cells or \( 10 \times 10^6 \) lymphocytes dispensed in 0.1 ml of Hanks’ solution and administered via a No. 27 gauge syringe needle into sites distributed over the mechanically clipped skin of the host’s dorsum and flanks. In some experiments samples of \( 20 \times 10^6 \) cell equivalents of antigenic extract in 0.1 ml of phosphate-buffered saline solution were employed as challenge antigen instead of viable intact cells. Several replicates of each inoculum and its controls were injected intracutaneously at randomly distributed sites. The lesions that developed were scored at 24-hr intervals on an arbitrary scale which took into consideration their diameter, inflammation, edema, induration, and necrosis, particular emphasis being put upon the extent of inflammation and induration: ±, 2-4 mm; 1+, 4-6 mm; 2+, 6-8 mm; 3+, 8-10 mm; 4+, 10-14 mm; and 5+, >14 mm. Scores of replicate reactions on panels of at least six similarly treated animals were pooled and their mean values, rounded off to the next highest half-point, are presented in the tables and figures.

Peripheral Blood Leukocyte Counts.—These were performed on blood which flowed from small incisions made in the ear.

Whole Body Irradiation.—Whole body irradiation involved exposure of animals to a cesium-137 source delivering 175 R/min. Monodisperse cell suspensions in Hanks’ solution were similarly irradiated in plastic Petri dishes. Additional special techniques will be described in the sections of the text to which they relate.

EXPERIMENTS AND OBSERVATIONS

Control Data.—To provide essential base lines it was necessary to define the kinetics of the various cutaneous reactions to standard cellular inocula as expressed in our particular strains of guinea pig.
**Direct reactions:** When animals of strains 2 and 13, presensitized against each others' tissue antigens, were challenged intradermally with suspensions of donor cells or antigenic extract they developed typical strong reactions at the inoculation sites, attaining peak intensities of about 3-4+ by 24 hr. The reactions persisted for up to 48 hr and then began to wane, subsidence being far advanced within 96 hr.

Fig. 1 A summarizes the courses of direct reactions in strain 2 guinea pigs sensitized against strain 13 tissue antigens and vice versa, and also indicates the levels of the nonspecific responses of these animals to control inocula of isologous cells. Inoculation of antigenic extract provoked larger skin lesions (16–18 mm in diameter) than node cells or lymphocytes.

**ILT reactions:** When lymphocytes or node cells from specifically presensitized donors of one strain were inoculated into the skins of hosts of the other strain (Fig. 1 B) or into (strain 2 × strain 13)F₁ hosts (Fig. 1 C) violent, highly indurated ILT reactions, 12–20 mm in diameter, were incited. These reactions also peaked at 24 hr, remained at maximal intensity for about 72 hr, and began to wane by 96 hr after inoculation. Firm, blanched nodules, often with necrotic centers, persisted at the sites for several days.

**NLT reactions:** NLT reactions are the local GVH-type reactions incited by inocula of immunocompetent cells from unsensitized donors in the skins.
of hosts that confront them with alien transplantation antigens. The courses of NLT reactions incited by strain 13 lymphocytes in the skins of strain 2 hosts and vice versa are summarized in Fig. 1 D. Relatively weak NLT reactions (2+) peaking by about 96 hr developed when lymphoid cells from either parental strain were injected into F1 hybrid recipients (Fig. 1 C).

Lymphocytes were more effective than node cells as incitors of NLT reactivity, the responses to node cells being slower to develop and tending to fade out more rapidly. As illustrated, NLT reactions are typically biphasic, an initial, low-level "first inflammatory episode" of prompt onset remaining relatively quiescent for about 2 days and then increasing to peak intensity over a 48-hr period, the so-called "flare-up" phase (2). When the subjects are members of an inbred strain instead of F1 hybrids, the flare-up may represent at least in part a direct hypersensitivity reaction on the part of the host to the antigenic stimulus afforded by the cellular inoculum (2, 4).

**Apparent Inducibility of Delayed Reactivity to Parental Strain Antigens in F1 Hybrid Hosts.**—When F1 hybrid hosts were rechallenged with normal, strain 2 lymph node cells 7 days after a previous inoculation with similar cells, intense (3-4+) reactions developed within 24 hr, having all the characteristics of delayed reactions to transplantation antigens in this species (Table I).

A third inoculation of the animals with normal lymphocytes from the original donor source precipitated a similar response. Similar results were obtained when node cells from normal strain 13 donors were injected into F1 hybrid animals. The immunologic specificity of this apparent reactivity against material associated with parental strain cells was evidenced by the finding that repeated inoculation of hybrid subjects with node cells from hybrid donors failed to elicit reactions. It was also found that when cell-free antigenic material of strain 13 origin was injected intracutaneously into hybrid animals, weak but definite delayed reactions were incited by the second and third challenges with this antigen.

This apparent sensitization of "genetically tolerant" F1 hybrid animals to

| TABLE I
| Results of Repeated Intradermal Injection of (Strain 2 \times Strain 13)F1 Hybrid Guinea Pigs with Lymph Node Cells or Antigenic Extract from Normal Parental Strain Donors |
| Origin and nature of inoculum | Reaction scores |
|-----------------------------|----------------|
|                            | 1st skin test at 90 hr | 2nd skin test at 24 hr* | 3rd skin test at 14 hr† |
| (Strain 2 \times 13)F1 cells | ±             | ±             | ±             |
| Strain 2 cells              | 2.5±          | 3+            | 4+            |
| Strain 13 cells             | 2+            | 3+            | 4+            |
| Strain 13 extract           | ±             | 2+            | 2+            |

* 2nd skin test was carried out 7 days after initial skin inoculation.
† 3rd skin test was carried out 14 days after initial skin inoculation.
an antigen associated with parental strain cells is difficult to explain. The results of skin-grafting tests were consistent with the homozygosity of each of our putative inbred strains, and there is little evidence to sustain belief in recessively determined transplantation antigens in any species (17).

To determine whether exposure of F1 hybrid guinea pigs to skin grafts from parental strain donors would also affect their reactivity to subsequent intradermal inocula of node cells of the same genetic origin, the following experiment was performed. Eight F1 hybrid animals bearing healthy skin grafts from each of their parental strains (one on each side of the trunk) were inoculated concomitantly with node cells from (a) a strain 2 anti-13 donor, (b) a normal strain 2 donor, (c) a strain 13 anti-2 donor, and (d) a normal strain 13 donor (Table II).

Whereas the immune cells consistently evoked strong ILT reactions within 24 hr, there was negligible reactivity at this time at the sites inoculated with cells from normal donors, though typical NLT reactions developed at these sites, attaining 2–3+ scores, during the following 3 days. These observations indicate, firstly, that previous exposure to skin grafts from normal parental strain donors did not induce reactivity to subsequent inocula of normal lymph node cells from the same donors, and, secondly, that the skin grafts were insusceptible to the reactivity which the host developed to lymphoid cells of

| TABLE II |
| Results of Repeated* Intradermal Inoculation of Normal (Strain 2 X Strain 13)F1 Guinea Pigs with Node Cells from Normal and Sensitized Parental Strain Donors |

| Hosts       | 24-hr reaction scores after challenge with node cells from the following donors |
|-------------|--------------------------------------------------------------------------------|
| Exp.        | Type                   | No. tested | Strain 2 anti-13 | Strain 13 anti-2 | Strain 2 | Strain 13 |
| 1           | (2 X 13)F1‡          | 8          | 1st test 3+      | 3+               | ±        | ±        |
|             |                       |            | 2nd test 4+      | 4+               | ±        | ±        |
|             |                       |            | 3rd test 4+      | 4+               | ±        | ±        |
| 2           | (2 X 13)F1            | 2          | 1st test 4+      |                  |          |          |
|             |                       |            | 2nd test         | 4+               |          |          |
|             |                       |            | 3rd test         |                  |          |          |
| 3           | (2 X 13)F1            | 2          | 1st test 4+      |                  |          |          |
|             |                       |            | 2nd test         | 4+               |          |          |
|             |                       |            | 3rd test         |                  |          |          |
| 4           | Strain 2              | 16         | 1st test 4+      |                  |          |          |
|             |                       |            | 2nd test         | +                |          |          |
|             |                       |            | 3rd test         | ±                | 4+       |          |
| 5           | Strain 13             | 9          | 1st test 4+      |                  |          |          |
|             |                       |            | 2nd test         | +                | ±        |          |
|             |                       |            | 3rd test         | 3+               | ±        | ±        |

* Skin tests were carried out on days 1, 7, and 14.
‡ These animals bore a well-established graft of skin from each parental strain.
the same parental strain origin, as evidenced by the continued well-being of these grafts.

When the same hosts were challenged with all four types of node cell suspension for a second or a third time it was found that the inocula from unsensitized donors also incited strong reactions within 24 hr (Table II). These reactions were completely indistinguishable from those incited by the sensitized cells.

If hybrid animals, previously inoculated with strain 2 anti-13 node cells were subsequently challenged with node cells from normal strain 13 donors, the latter inocula also incited strong (4+) 24-hr reactions as did a second challenge 14 days later with cells from normal strain 2 donors. Similar results were obtained when the first donor was a strain 13 animal sensitized against strain 2 antigens and the donor of the subsequent normal cell inoculum belonged to strain 2.

The results of experiments 4 and 5 (Table II) serve to indicate that these unexpected observations cannot be ascribed to the development of a totally nonspecific hyperreactive state to any kind of intradermal inoculum. Isologous lymphoid cells injected into inbred strain hosts which had received homologous cells failed to provoke local skin reactions.

A possible explanation for the apparent cross-reactivity observed in the F₁ animals is that immunologic effector cells of donor strain origin from the initial test inoculum persisted in a reactive condition and were largely responsible for the reactivity observed when the hosts were subsequently challenged with cells from a normal donor of the other parental strain.

Recall Flare Reactions.—Strain 2 guinea pigs which had been injected intradermally at multiple (usually four to eight) sites with samples of 7.5–10 × 10⁴ lymphocytes from strain 13 donors, and subsequently manifested NLT reactions, were injected intravenously 1 wk later with 50 × 10⁶ cell equivalents of strain 13 antigenic extract. In five of eight subjects delayed reactions of dramatic intensity developed at some or all of the sites of the previous NLT reactions. The new lesions sometimes exceeded 20 mm in diameter, the induration associated with them usually involved the integument to a depth of 7 mm, there was considerable central necrosis, and the healing process took upwards of 5 days for completion (Table III).

This interesting local cutaneous response to the systemic readministration of antigenic material to which the subject was already sensitized was unpredictable with regard to its sporadic occurrence in individual animals and the fact that it usually developed only at some of the recipient’s previous NLT reaction sites. It is noteworthy that in two animals in which only weak recall flares developed there was an associated intense, generalized inflammation of the entire skin which persisted for 24 hr. Once an animal had responded by a recall flare, after intravenous injection with antigen, it proved completely refractory when rechallenged by the same route 7, 14, or 21 days later.
### TABLE III

*Recall Flare Reactions Incited by Cutaneous Inoculation of Antigenic Extract into Guinea Pigs Sensitized by the Intradermal Route*

| Host          | Pretreatment of host | Mean NLT reaction scores | Origin of intravenously injected antigenic extract | No. of sites reactivated per host | Recall flare reaction scores | Duration of reactions | Days |
|---------------|----------------------|--------------------------|---------------------------------------------------|---------------------------------|-----------------------------|-------------------------|------|
| Strain 2      | Skin tested with     | +++                      | Strain 13                                         | 2/4                             | 3+,3+                       |                         | 9,6  |
|               | strain 13 lymphocytes|                          |                                                   |                                 |                             |                         |      |
|               | "                     | "                        |                                                   |                                 |                             |                         |      |
|               | "                     | "                        |                                                   |                                 |                             |                         |      |
|               | "                     | "                        |                                                   |                                 |                             |                         |      |
|               | "                     | "                        |                                                   |                                 |                             |                         |      |
|               | "                     | "                        |                                                   |                                 |                             |                         |      |
| Strain 13     | Skin tested with     | 2+                       | Strain 2                                         | 0/4                             |                             |                         |      |
|               | strain 2 lymphocytes  |                          |                                                   |                                 |                             |                         |      |
|               | "                     | "                        |                                                   |                                 |                             |                         |      |
|               | "                     | "                        |                                                   |                                 |                             |                         |      |

This inability to give a second recall flare response, and the failure of some subjects to give a primary recall flare, may have resulted from the development of appreciable titers of "blocking" or enhancing isoantibodies (18) in hosts as a consequence of exposure to the initial intracutaneous inocula of strain 13 cells which incited the NLT reactions. However, this explanation cannot account for the development of recall flares at only some of the previous NLT reaction sites in some animals. These recall flares are reminiscent of those observed by Rapaport and Converse (19) at the sites of rejection of previous skin homografts in human volunteers rechallenged with skin from their original donors. Silverstein's (20) findings in rabbits sensitized by the intraocular route to a heterologous serum protein and reexposed to this antigen by the intravenous route are pertinent here. It seems likely that our recall flares were incited by interaction of host immunological "memory" cells persisting at the cutaneous sites of the NLT reactions (after the extinction of these reactions by host-versus-graft reactivity), with the strain 13 antigen introduced into the subject's bloodstream.

### Influence of Whole Body Irradiation on Leukocyte Count

Since Brent and Medawar (2, 4) gave no data concerning the leukocyte counts in the irradiated outbred Hartley guinea pigs they used to study transfer reactions, we have determined the influence of whole body irradiation over the range 600–2500 R on the peripheral leukocyte counts of strain 2 and strain 13 animals. Each animal was bled immediately after irradiation, and at regularly spaced intervals thereafter, and total and differential counts were made. Since the responses of both strains to irradiation were similar, the results have been pooled (Table IV and Fig. 2). Animals that received 600 R or more succumbed within 8–15 days; those which received 2500 R lived for 5–7 days.
The findings show that to reduce the leukocyte count to $0.5 \times 10^6$ cells/ml or below, within 96 hr, requires at least 2000 R. At all levels of irradiation the differential counts showed a severe lymphocytopenia within 24 hr followed by a progressive granulocytopenia which attained a low level by 96 hr.

**TABLE IV**

*Granulocyte and Lymphocyte Counts (× 10⁶ Cells/ml) at Stated Intervals after Various Doses of Whole Body Irradiation*

| Time after irradiation | 600 R | 1000 R | 1500 R | 2000 R | 2500 R |
|------------------------|-------|--------|--------|--------|--------|
| Lymphocyte             |       |        |        |        |        |
| Granulocyte             |       |        |        |        |        |
| 0 hr                   | 4.05  | 3.9    | 3.4    | 4.1    | 4.1    |
|                        | 5.32  | 5.15   | 5.1    | 5.9    | 5.0    |
| 18 hr                  | 3.8   | 2.2    | 2.1    | 2.65   | 10.0   |
|                        | 7.4   | 7.1    | 10.0   | 1.5    | 12.0   |
| 24 hr                  | 1.4   | 1.3    | 0.85   | 0.37   | 0.15   |
|                        | 2.0   | 6.3    | 0.6    | 2.72   | 7.65   |
| 48 hr                  | 1.1   | 0.60   | 0.37   | 0.38   | 0.22   |
|                        | 2.7   | 3.17   | 2.72   | 3.95   | 6.7    |
| 72 hr                  | 0.73  | 0.6    | 0.36   | 0.17   | 0.31   |
|                        | 2.95  | 2.0    | 1.45   | 1.2    | 2.8    |
| 96 hr                  | 0.63  | 0.41   | 0.37   | 0.13   | 0.22   |
|                        | 2.05  | 0.82   | 0.72   | 0.35   | 0.27   |
| 120 hr                 | 1.1   | 0.45   | 0.23   | 0.11   | 0.17   |
|                        | 2.35  | 0.75   | 0.27   | 0.19   | 0.08   |
| 6 days                 | 0.77  | 0.24   | 0.14   | 0.16   | 0.14   |
|                        | 0.98  | 0.48   | 0.16   | 0.6    | 0.16   |
| 7 days                 | 0.74  | 0.33   | 0.17   | 0.20   | 0.31   |
|                        | 0.45  | 0.17   | 0.17   | 0.2    | 0.31   |
| 8 days                 | 0.43  | 0.37   | 0.088  | 0.112  | 0.08   |
|                        | 0.37  | 0.088  | 0.112  | 0.08   | 0.08   |

Median survival time (days): $13.2 \pm 1.15$, $10.0 \pm 0.8$, $9.0 \pm 0.7$, $6.7 \pm 0.96$, $5.7 \pm 0.33$

**Fig. 2.** Total leukocyte count (---) and lymphocyte count (-----) in strain 2 and strain 13 guinea pigs exposed to 600 and 1500 R respectively.
In general, animals exposed to 1000 R or more had become very weak by the time their total leukocyte counts had dropped below $1 \times 10^6$ cells/ml and they did not survive for more than an additional 2–4 days. By contrast, animals which received 600 R were much healthier when their leukocyte counts had fallen below $1 \times 10^6$ cells/ml and they invariably survived for another 4–7 days.

Influence of Whole Body Irradiation on Capacity to Give Direct Reactions.—To elucidate the relationship between a sensitized guinea pig's leukocyte count and its capacity to give a direct reaction, two experiments were performed. In the first, panels of sensitized animals were exposed to 600, 1000, or 1500 R respectively and challenged, 24 hr later, with donor antigenic material in the form of lymphocytes, node cells, and antigenic extracts. All of the subjects responded by direct reactions of at least 4+ intensity to the three test antigens used. However, it is noteworthy that these reactions were much more striking in appearance than those in unirradiated subjects. Not only was the intensity of the inflammatory component greater, but it also extended beyond the area of induration. Furthermore, the lesions persisted longer and there was frequently some necrosis. Very little necrosis results from direct reactions in unirradiated animals and the lesions fade away faster. The influence of irradiating the host on the qualities of the direct reaction is similar to its influence on those of NLT reactions, as observed by Brent and Medawar (2).

In the second experiment six strain 2 guinea pigs sensitized against strain 13 antigens received 600 R whole body irradiation and peripheral leukocyte counts were made daily. At some time, as each animal became leukopenic, it was challenged intradermally with strain 13 lymphocytes as antigen. The results (Fig. 3) indicate that below a threshold concentration of leukocytes a guinea pig's capacity to express direct reactivity becomes attenuated, very feeble reactivity being associated with a count of $0.3 \times 10^6$ cells/ml.

![Fig. 3. Direct reactions to inocula of strain 13 lymphocytes (as antigen) in strain 2 guinea pigs exposed to 600 R and whose leukocyte counts had fallen to the levels indicated per milliliter when tested.](image-url)
Influence of Irradiation on a Host's Capacity to Give an ILT Reaction.—When normal parental or F₁ hybrid subjects were exposed to 600, 1000, or 1500 R and inoculated intradermally, 24 hr later, with lymphocytes or node cells from appropriate sensitized donors, strong (4+) transfer reactions developed within 24 hr. These reactions persisted longer and more necrosis was associated with them than in unirradiated hosts.

In a second experiment inoculation of the irradiated hosts with immune lymphoid cells was delayed until their total leukocyte counts had fallen below $1.5 \times 10^6$ cells/ml. The results (Table V) indicate that a certain minimal or threshold leukocyte count is essential for the development of significant ILT reactions. This was particularly evident when node cells rather than lymphocytes were used as the incitants. Indeed it will be noted that at low concentrations of leukocytes in the bloodstream, inocula comprised of lymphocytes still incited weak ILT reactions when node cells were ineffective. Again, the transfer reactions which developed in this group of irradiated animals were slow to develop, intensely inflamed, and soft in consistency as a consequence of edema.

If peripheral leukopenia is responsible for the impaired capacity of these irradiated guinea pigs to develop ILT reactions, then addition of host-type cells to the immune donor lymphoid cell suspensions before intracutaneous injection should make good the deficit and allow these reactions to develop (8, 21). This prediction was borne out: when inocula, comprised of $7.5 \times 10^6$ strain 2 anti-13 lymphocytes mixed with $7.5 \times 10^6$ strain 13 or F₁ node cells (as “supplementing” antigen) in 0.1 ml of Hanks' solution, were introduced into the skins of irradiated and highly leukopenic strain 13 hosts, ILT reactions of up to 3.5+ peak intensity developed.

### Table V

**ILT Reaction Scores in Normal and in Irradiated (600 R) Highly Leukopenic Guinea Pigs**

| Donor of immune cells | Host | Treatment of host | Leukocyte count $\times 10^6$ cells/ml at time of skin test | Peak transfer reaction scores induced by | Lymph node cells | Lymphocytes |
|-----------------------|------|-------------------|-----------------------------------------------------------|------------------------------------------|-----------------|------------|
| Strain 13 anti-2      | Strain 2 |             | 13.6                                                      | 3.5+                                    | 4+              |            |
| Strain 13 anti-2      | Strain 2 | 600          | 1.3                                                       | 1.5+                                    | 2+              |            |
| Strain 13 anti-2      | (2 × 13)F₁ |             | 12.4                                                      | 3+                                      | 4+              |            |
| Strain 13 anti-2      | (2 × 13)F₁ | 600          | 0.9                                                       | +                                       | 2+              |            |
| Strain 2 anti-13      | Strain 13 |             | 8.0                                                       | 4.5+                                    | 5+              |            |
| Strain 2 anti-13      | Strain 13 | 600          | 0.3                                                       | ±                                       | ±               |            |
| Strain 2 anti-13      | (2 × 13)F₁ |             | 12.4                                                      | 4+                                      | 4.5             |            |
| Strain 2 anti-13      | (2 × 13)F₁ | 600          | 0.9                                                       | ±                                       | 2+              |            |
| Strain 13 anti-2      | Strain 13 |             | 11.2                                                      | ±                                       | ±               |            |
| Strain 2 anti-13      | Strain 2 |             | 12.8                                                      | ±                                       | ±               |            |
**Influence of Irradiation on a Host's Capacity to Give an NLT Reaction.**—When strain 2 or strain 13 guinea pigs were exposed to 600 or 1000 R and inoculated intradermally with normal lymphocytes 24 hr later, NLT reactions developed but their tempo was much slower than in unirradiated hosts (Fig. 4). Peak intensities (which were slightly higher than in normal subjects, see Fig. 1 D) were not reached until 5–6 days instead of about 4 days after inoculation. It will be noted that whereas rather feeble ILT reactions were incitable by strain 2 cells in normal strain 13 hosts, much stronger reactions developed in preirradiated hosts. As with ILT reactions, tests were made to evaluate the significance of the host leukocyte count for the development of NLT reactions.

![Figure 4](image_url)

**Fig. 4.** NLT reactions incited by inoculation of strain 13 lymphocytes (●—●) and lymph node cells (○—○) into strain 2 hosts exposed to 600 R 24 hr previously, and by strain 13 lymphocytes (●—●) and lymph node cells (○—○) into strain 13 hosts exposed to 600 R 24 hr beforehand.

Five normal strain 2 animals which had been given 600 R 1, 3, 5, 7, and 9 days beforehand respectively, and whose leukocyte counts ranged from 0.5 to 8.9 \times 10^6 cells/ml, were challenged intradermally with node cells from a normal strain 13 donor. The results (Fig. 5) revealed that the intensities of the NLT reactions which developed were dependent upon the concentration of leukocytes in the host’s blood. Subjects whose counts had fallen to 2.3 \times 10^6 cells/ml or below failed to express the flare-up stage of the reaction, and when the count was below this level the host was virtually incapable of expressing even the first inflammatory episode of the NLT reaction.

These findings indicate that host peripheral leukocytes are essential for the development of both ILT and NLT reactions in guinea pigs. The strong presumption is that these cells are trapped from the circulation at the intracutaneous inoculation sites where they function as the antigen with which the donor cells interact.
TRANSFER REACTIONS IN GUINEA PIGS

Evaluation of Active Immune Participation of the Host in ILT Reactions.—When homozygous animals instead of F₁ hybrids are used as hosts for NLT reactions, apart from providing antigenic material in the form of peripheral leukocytes, the possibility remains that they themselves become sensitized to the alien attacking cells so that an active host-versus-graft reaction is eventually superimposed upon the initial GVH reactivity. By exposure of their hosts to 600 R 24 hr before cell transfer Brent and Medawar (4) felt that they had eliminated this complication.

To evaluate the influence of previous irradiation on a guinea pig's capacity to become sensitized after exposure to tissue antigens, three strain 2 guinea pigs were exposed to 600 R and inoculated intradermally 24 hr later with lymphocytes from normal strain 13 donors. These incited NLT reactions as anticipated. 3 days after irradiation each animal was challenged with two kinds of inocula of strain 13 origin: (a) node cells and (b) antigenic extract. All three animals responded by typical (3-4+) delayed reactions to these inocula (see Fig. 6), indicative of their active sensitization to the initial strain 13 cell inocula. The peripheral leukocyte counts in these animals 24 hr after the second skin challenge, and at the time when their direct reactions were of peak intensity, ranged from 3.4 to 3.8 X 10⁶ cells/ml.

Finally, 8 days after the initial skin testing (and 9 days after irradiation) when their peripheral leukocyte count was 0.3 X 10⁶ cells/ml, these animals were rechallenged with node cells and antigenic extract from the donor strain. This time they failed to respond, presumably because of the paucity of immunologic effector cells in their bloodstream. If, as Brent and Medawar (2, 4) have argued, the NLT reaction in an irradiated guinea pig is entirely a GVH reaction, and the flare-up stage is the consequence of donor cell proliferation without host participation, typical NLT reactions should have developed at
Fig. 6. Direct reactivity in strain 2 guinea pigs resulting from intradermal inoculation of strain 13 lymphocytes 24 hr after exposure to 600 R. Strong (3-4+) reactions were incitable by both strain 13 node cells and antigenic extract. Note that rechallenge of the hosts with strain 13 antigenic material 192 hr after primary sensitization (and 216 hr after irradiation) failed to incite reactions.

the sites of secondary and tertiary inoculation of node cells in the present experiment.

Influence of In Vitro Irradiation on Subsequent Immunologic Performance of Lymph Node Cells In Vivo.—The experiments now to be described were designed to complement those described in the previous section by elucidation of the immunologic and antigenic potential of lymph node cells after irradiation in vitro. Node cells from normal strain 13 animals and from similar guinea pigs presensitized against strain 2 tissue antigens were exposed to 2000 R in vitro, washed, and counted. This level of irradiation did not immediately prejudice the viability of the cells, as evidenced by the results of trypan blue dye exclusion tests. Samples of these cells at appropriate dilutions were injected into panels of normal and irradiated (600 R 24 hr previously) strain 2 guinea pigs.

The results, some of which are presented graphically in Fig. 7, show that irradiation of a population of sensitized cells does not inhibit its capacity to incite an NLT reaction in an irradiated host. However, the reactions were weaker and of shorter duration than those elicited by comparable doses of unirradiated cells (see Fig. 1). Irradiation in vitro of node cells from normal donors did not impair their ability to develop the first stage of the NLT reaction, though it did prevent the development of the flare-up phase.

Among possible explanations for this abrogation of the principal component of the NLT reaction are the following. Irradiation (a) abolished the capacity of the transferred lymph node cells to divide, (b) weakened the capacity of the cells to sensitize their host, so that its immunologic contribution to the NLT reaction was greatly reduced, and (c) affected lymphocytes by greatly decreasing their life expectancy, thus interfering with their availability at skin test sites.
Fig. 7. Incitement of cutaneous hypersensitivity reactions by inoculation of lymphoid cells irradiated in vitro into preirradiated hosts. (a) ILT reaction incited by inoculation of strain 13 node cells, exposed to 2000 R in vitro, from a specifically presensitized donor into the skin of a strain 2 host exposed to 600 R 24 hr beforehand (O—O). (b) Incomplete NLT reaction incited by inoculation of irradiated node cells from a normal strain 13 donor into a preirradiated strain 2 host (O—O). Note the absence of the inflammatory phase. (c) Typical direct reaction incited by inoculation of strain 13 node cells as antigen into strain 2 hosts which, 24 hr after exposure to 600 R, were inoculated with strain 13 node cells which had been exposed to 2000 R in vitro (∆—∆).

as “targets” for the host-versus-graft response when it develops. The following experiments were designed to discriminate between these possibilities:

(a) A normal and a preirradiated (600 R 24 hr previously) strain 2 guinea pig were injected intracutaneously with a total of 80 X 10⁶ irradiated (2000 R) normal strain 13 node cells, at a number of sites. 3 days later each animal was challenged with strain 13 node cells (as antigen). Both animals responded by strong direct reactions leaving no doubt as to the antigenic efficacy of the irradiated cells and of the capacity of the irradiated hosts to become sensitized (Fig. 7).

(b) The nodes and spleens from two strain 2 guinea pigs hyperimmunized against strain 13 tissue antigens were harvested; a cell suspension was prepared and divided into two equal portions. One portion was irradiated in vitro, the other was not. Each portion (comprising 370 X 10⁶ viable cells in 5 ml of Hanks’ solution) was inoculated intraperitoneally into a normal strain 2 guinea pig. 5 days later, when these animals were challenged intradermally with strain 13 node cells (as antigen), only the recipient of the unirradiated cells gave a direct reaction, suggesting that the transferred irradiated cells were ineffective in mediating sensitivity at this time.

Further Experiments to Determine the Importance of Skin Cells and Peripheral Leukocytes as Principal Host Reactants in Lymphocyte Transfer Reactions.—(a) Four F₁ hybrid guinea pigs each received two large, rectangular, partial thick-
ness trunk skin grafts, approximately 3.5 × 4.0 cm, one from each parental strain, transplanted to full thickness beds on opposite sides of the thorax. Subsequently, when these grafts were well established, node cells from strain 2 anti-13 and strain 13 anti-2 donors were injected into the three genetically different kinds of skin available on these animals, i.e. the animal's own (F1 hybrid) skin, and skin of graft origin from each parental strain. Strong ILT reactions were incited in all three types of skin by both types of node cell inocula, irrespective of whether the affected skin was genetically compatible with the inoculum (Table VI). Although the transfer reactions in the grafts were impressive, in some instances involving their entire extent, they took slightly longer to develop than those in the F1 host's own skin. These findings lend no support to the thesis that constitutive skin cells are significant as antigen in ILT reactions.

| Donor of node cell inoculum | Type of skin tested | Intensity of skin reactions at 24 hr | 48 hr |
|-----------------------------|--------------------|------------------------------------|------|
| Strain 2 anti-13            | (2 × 13)F1         | 4+                                 | 5+   |
| Strain 2 anti-13            | Strain 2           | 3+                                 | 4+   |
| Strain 2 anti-13            | Strain 13          | 3+                                 | 5+   |
| Strain 13 anti-2            | (2 × 13)F1         | 4+                                 | 4+   |
| Strain 13 anti-2            | Strain 13          | 2+                                 | 3+   |
| Strain 13 anti-2            | Strain 2           | 3+                                 | 3+   |

(b) Four strain 13 animals received 500 R, followed 1 day later by a "rehabilitating" intravenous infusion of 800 × 10⁶ lymphoid and marrow cells in Hanks' solution from F1 hybrid donors. 3 wk later, when their peripheral leukocyte count had returned to normal, these now chimeric animals were inoculated intradermally with strain 13 anti-strain 2 node cells. Within 48 hr violent ILT reactions of 4-5+ intensity developed at all test sites. Since the only source of strain 2 antigen-bearing cells with which the strain 13 cells could interact were F1 leukocytes in the host's blood, this finding strongly supports the premise that when transfer reactions take place in normal hosts the principal host reactants are cells of hematologic origin.

DISCUSSION

A study has been made of the capacity of guinea pigs to give direct ILT and NLT reactions at various times after exposure to whole body X-irradiation, when their leukocyte counts had fallen to known levels, to evaluate the possible significance of these cells rather than skin cells as reactants. Irrespective of whether they had received 600, 1000, or 1500 R 24 hr beforehand, spe-
cifically presensitized animals of the one strain challenged with either viable lymphoid cells or antigenic extracts from donors of the other strain responded by strong direct reactions. However, delay of skin testing until the host’s leukocytes had fallen to $3.7 \times 10^5$ cells/ml revealed that, below a certain threshold level, the intensity of the reactions incited depended upon the host’s leukocyte concentration at the time of challenge. Even with counts as low as $0.3 \times 10^5$ cells/ml a feeble though significant level of reactivity was detectable (8, 22). These findings are in accord with the established role of lymphocytes as mediators of transplantation hypersensitivity and evidence that immunologically committed lymphocytes are more radioresistant than uncommitted lymphocytes (23).

When ILT tests were carried out 24 hr after irradiation, strong reactions were incited but when longer delays were involved the intensities of the host’s responses began to decline with the peripheral leukocyte counts, only very weak reactions being incitable when the counts had fallen below $1.0 \times 10^5$ cells/ml at the time of cell transfer. These observations constitute a prima facie case that peripheral leukocytes rather than skin cells are the principal host target cells with which the transferred immunocompetent cells engage immunologically in ILT reactions. Additional findings that sustain this premise are as follows:

(a) Preirradiated and highly leukopenic hosts were capable of expressing strong ILT reactions if the putative donor “attacking” cell population was mixed with a population of host-type or F1 lymphoid cells (antigenic supplementation) before transfer. This observation is particularly important since it refutes the obvious possibility that irradiation per se debilitates the hosts to the extent that they become totally incapable of expressing skin reactions.

(b) Node cells from strain 2 animals sensitized against strain 13 antigens and injected into long established strain 2 skin grafts on (strain 2 X strain 13)F1 hosts incited ILT reactions that were just as intense as those incited by similar inocula in either the host’s own, i.e. (F1) skin, or in established strain 13 skin grafts.

(c) Strain 13 animals exposed to 500 R whole body irradiation and rehabilitated (and made chimeric) by transfusion of lymphohematopoietic cells from F1 hybrid donors responded by strong ILT reactions to challenge with strain 13 anti-2 lymph node cells.

ILT reactions were incitable by inoculation of strain 2 lymphoid cells into strain 13 hosts exposed to 600 or 1000 R 24 hr previously, or vice versa. However, as other investigators have reported, the reactions were slower in tempo and attained slightly higher peak intensities. Delay of skin testing after host irradiation gave evidence of a direct relationship between the peak intensities of the reactions incited and the host’s leukocyte concentration at the time of the skin test. Animals whose counts had fallen to $2.3 \times 10^5$ cells/ml failed to develop the flare-up stage of the reaction, and those with lower counts gave no
significant responses. Indeed, it is questionable whether they gave even the initial recognition phase. Thus ILT and NLT reactions in the guinea pig resemble those in other species in that the target cells are of hematologic origin.

Qualitatively all three types of cutaneous reactivity studied in irradiated subjects differed markedly in their expression from equivalent reactions expressed by unirradiated animals. The intensity of the inflammatory component was greater in the former, it extended beyond the area of induration, the lesions persisted longer, and there was more necrosis. Although direct reactions developed with normal promptitude in irradiated animals, ILT and NLT reactions were slower to develop, possibly because of an increased time taken to trap the necessary number of cells from the host's bloodstream. Reduction in the irradiated host's platelet count and increased permeability and fragility of its fine cutaneous blood vessels may have been responsible for the enhanced lesions or reactions which developed; i.e., irradiation may simply have increased the "sensitivity" of the recording milieu.

The finding that guinea pigs exposed to 600 R and inoculated intracutaneously with homologous donor lymphoid cells 24 hr later responded by typical intense direct reactions to challenge with antigenic material 3 days later, i.e. 4 days after irradiation, (a) confirms the promptitude with which delayed hypersensitivities can be called into being and (b) reinforces a recent report that exposure of guinea pigs to lethal doses of irradiation neither abrogates nor delays their ability to develop high levels of homograft hypersensitivity (24).

In the light of these observations it is probable that the flare-up phase of the NLT reaction in both normal and irradiated homozygous guinea pigs is predominantly a direct reaction on the part of the host in which the transferred alien lymphocytes that initiated the NLT reaction now play the role of antigen. The original interpretation of the flare-up as being predominantly an expression of an antigen-stimulated, proliferative process on the part of the transferred cells is inconsistent with all other examples of GVH reactivity, and virtually devoid of sustaining evidence (24). If the present interpretation is correct, the "fade-out" of an NLT reaction reflects degeneration of the cellular antigens that incited the reaction. The problem remains of accounting for the flare-up phase and fade-out of NLT reactions in normal or irradiated genetically tolerant F1 hybrid hosts incited by lymphocytes from one or other of their parental strain donors.

SUMMARY

Using guinea pigs of strains 2 and 13 and their F1 hybrids as experimental subjects, various lines of evidence have been obtained that in this species, as in all others tested, the only significant cellular antigens with which donor lymphocytes engage when normal and immune lymphocyte reactions are incited are radiosensitive leukocytes. Constitutive cells of the skin are unimportant.
(a) The intensities of these reactions in irradiated subjects are dependent upon the peripheral leukocyte concentration. When this falls below a certain threshold no reactions are incitable.

(b) Highly leukopenic animals are capable of developing immune lymphocyte transfer (ILT) reactions if normal lymphoid cells of their own genetic constitution are mixed with the putative attacking donor cells, as "supplementing antigen," before inoculation.

(c) Radiation-chimeric strain 13 animals having F1 hybrid leukocytes in their bloodstream give typical ILT reactions when challenged intradermally with strain 13 anti-2 node cells.

Exposure of strain 2 animals to 600 R does not prevent their becoming actively immunized if, 24 hr later, they are injected intradermally with strain 13 lymphocytes. However, this sensitization, revealed by the host's capacity to give delayed hypersensitivity reactions, wanes as leukopenia progresses. On the basis of this and other findings it is argued that the flare-up stage of the NLT reaction in preirradiated hosts is mainly an expression of host sensitivity against the transferred alien cells.

Two unexpected observations have been made in the course of this study: (a) F1 hybrid animals developed what appeared to be a strong delayed hypersensitivity after intradermal inoculation with parental strain lymphoid cells or antigenic extracts prepared from them. (b) If strain 13 guinea pigs which had been sensitized against strain 2 tissue antigens by intradermal injection of lymphocytes 7 days beforehand were inoculated intravenously with strain 2 antigenic extract a significant proportion of the animals developed severe delayed necrotizing reactions, recall flares, at some or all of the healed skin inoculation sites.

The authors are indebted to Dr. J. Wayne Streilein for helpful advice and criticism of the manuscript and to Dr. Bruce A. Christie and Mr. George Sawchuck for expert assistance. The expenses of the work were defrayed in part by U.S. Public Health Service Grants AI-07001 and AI-10678. Dr. Zakarian was supported by a U.S. Public Health Service Postdoctoral Fellowship and an I. S. Ravdin, Mead Johnson Pennsylvania Plan Scholarship.

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