DIFFERENTIATION OF T-CELL PRECURSORS IN NUDE MICE DEMONSTRATED BY IMMUNOFLUORESCENCE OF T-CELL MEMBRANE MARKERS

BY F. LOOR AND B. KINDRED

(From the Basel Institute for Immunology, CH-4058 Basel, Switzerland)

(Received for publication 12 July 1973)

Among many deficiencies, congenitally thymus-less mice (nude mice) are lacking the T-cell compartment of the lymphoid system (1-3). However, by the use of chromosome marker studies, Wortis et al. (4) showed that bone marrow cells from nude mice were able to repopulate the thymus-dependent areas in irradiated mice. Thus, they concluded that nude mice have the T precursor cells but are deficient in the thymic epithelium necessary for their development.

It is interesting that one T-cell function can be restored by grafting a neonatal thymus from a normal mouse to a nude, i.e., the ability to reject skin graft from a third mouse strain (4, 5). However, as skin grafts of thymus donor strain are not rejected (6), one can be suspicious about the origin of the reactive T cells in these thymus-grafted mice. Thus, it is essential to find whether there is any significant host contribution to the whole T-cell population and whether such host-derived T cells could be fully physiologically active T cells.

The recent finding by Pritchard and Micklem (6) that the majority of the cells undergoing mitosis in a grafted thymus were of host type (as identified by the T6 chromosome marker) strongly suggests that functional host T cells can develop in a grafted neonatal thymus. However, the characterization of cells by that marker is tedious and does not allow one to distinguish between B and T cells.

With backcrossed nudes, lymphocyte surface antigens specific for host or graft donor strains become an excellent tool for the identification of the origin of lymphocytes found in the grafted animal. The immunofluorescence technique is simple and a large number of cells can be checked. Since H-2 antigens show heterogeneity of expression in a lymphocyte population and it is not possible to distinguish between T and B cells on the basis of H-2 antigens, we have avoided the use of these markers although the private H-2 specificities would make it possible to characterize any strain of mice. We, therefore, concentrated on a host-donor strain combination that allows easy identification of the origin of the T cells found in the grafted animal: AKR and BALB/c. Theta antigen (θ)1 is completely different in AKR (θ-AKR) and in BALB/c (θ-C3H) (7) and it can be easily detected by immunofluorescence (8). In the strain combination used, the thymocyte marker (thymus leukemia alloantigen [TL]) is also useful as BALB/c thymocytes express the antigen TL2, while no TL antigen at all can be

1 Abbreviations used in this paper: BSA, bovine serum albumin; FITC, fluorescein isothiocyanate conjugated; Ig, immunoglobulin; PBS, phosphate-buffered saline; R× MyG, polyvalent rabbit antibody against mouse immunoglobulins; TL, thymus leukemia alloantigens; TRITC, tetramethylrhodamine isothiocyanate conjugated; θ, theta antigens.
demonstrated on AKR thymocytes (9). The difference between BALB/c and AKR in TL antigens can also be demonstrated by immunofluorescence (Loot, F., and J. R. Little, unpublished data).

The specific strain combination used in our experiments was normal AKR mice and nu/nu mice backcrossed to a BALB/c background. The θ-marker was used for identification of T-cell origin in all lymphoid organs and the TL marker was also used in the thymus.

Data are presented that demonstrate that T cells of nude origin can repopulate an allogeneic thymus and migrate to other lymphoid organs.

Materials and Methods

Antisera and Fluorescent Conjugates.—Rabbit antimouse IgG (RαMγG) serum was used as a source of polyvalent antimouse immunoglobulin (Ig) antibody. The Ig fraction was purified and coupled to either fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TRITC) (Baltimore Biological Laboratories, Baltimore, Md.) as described (10); the conjugates will be referred to as TRITC RαMγG and FITC RαMγG.

AKR anti-θ-C3H serum and C3H anti-θ-AKR serum were generous gifts from Dr. G. Roelants. They were raised by the method of Raff (11), i.e., by immunizing AKR and C3H mice with thymus cells from C3H or AKR, respectively. AKR anti-θ-C3H serum was extensively absorbed on lymph node, spleen, and thymus cells from AKR mice, and C3H anti-θ-AKR serum was similarly absorbed on cells from BALB/c and C3H.

Anti-TL antiserum was a generous gift from Dr. J. R. Little. It was prepared by injecting TL+ leukemic cells (ASL1 with the TL specificities 1.2 and 3) from A/J mice into the congenic strain A-TL-. Further details about the characteristics of this serum will be published later (F. Loot and J. R. Little). For this particular experiment, anti-TL serum was absorbed with spleen and lymph node cells (TL- organs) from BALB/c, C57BL/6, and AKR mice, and with thymus cells from the TL- strains (C57BL/6 and AKR).

All of these mouse antisera were further submitted to 100,000 g centrifugation for 5 h to remove cell debris and aggregated immunoglobulins as well as fat. Normal sera from AKR, C3H, and A-TL- mice were treated similarly. All sera were diluted five times with 0.5% bovine serum albumin (BSA) in RPMI medium, pH 7.2, containing 10 mM NaN₃.

Direct Labeling of Mouse Antibodies with Fluorochromes.—Mouse immunoglobulins were first precipitated by (NH₄)₂SO₄ 1.6 M (final molarity 0°C), from serum that had been previously adsorbed as described above to make it fully specific, washed twice with the same medium, and redissolved in saline; they were then precipitated again by 17% Na₂SO₄. They were finally dissolved in saline and freed from sulfate by extensive, continuous dialysis against saline and then against sodium barbital buffer 0.054 M, pH 8.6. Insoluble proteins were removed by centrifugation. The immunoglobulin (soluble fraction) was used at a concentration of at least 5 mg/ml for block agarose electrophoresis; blocks of 20 X 50 cm were used with 0.5% agarose in sodium barbital buffer for the proteins from 10 ml of serum applied in 0.5% agarose-buffer. The solution was placed in a slit 1 cm wide cut 19 cm from the anode end, electrophoresis run at 600 V for 48 h at 0°C.

After this run, agarose slices 1 cm wide were then cut every centimeter and numbered +1 to +5 for slices cut towards the anode and −1 to −10 for slices cut towards the cathode, then put into individual centrifuge tubes with 10 ml of phosphate-buffered saline (PBS) and frozen. After thawing, most of the gel was broken down and the supernatant liquid was collected by centrifugation. This freeze-thawing procedure was repeated twice with 10 ml of PBS. Pooled supernatants from single tubes were concentrated on Diaflo Membranes UM2 (Amicon Corp., Redwood City, Calif.) until a protein concentration of at least 0.5 mg/ml was obtained. Pro
tein concentration was estimated by $E_{	ext{abs}}^{1.0}$ of 13.0 at 280 nm. Microzone electrophoresis was used to check the homogeneity of the fractions. The conjugation of the IgG fraction with TRITC was done by an original method proved to be efficient for lectins and chicken antibodies (F. Loor, unpublished data). The individual Ig fractions (from +5 to −10) were dialyzed against 10 times their volume of saline containing the fluorochrome at a ratio of 300 μg TRITC/mg protein or 125 μg FITC/mg protein. The pH was kept as close as possible to 8.5−8.7 with diluted NaOH for 2 h at 0°C. Dialysis against the TRITC or FITC solution was prolonged overnight at 0°C while leaving the pH to decrease. Unbound fluorochrome was removed by extensive continuous dialysis against PBS, pH 7.2. Conjugated immunoglobulins were then adsorbed on acetone-prepared mouse liver powder and freed of aggregates by ultracentrifugation. They were checked for specific activity. Usually electrophoretic fractions “−3” to “−6” gave a convenient fluorochrome to immunoglobulin molecule ratio, leaving full activity and good detection, and did not need further adsorption or reelectrophoresis to separate hypo- and hyperconjugated proteins.

**Membrane Immunofluorescence Technique.**—The general technique has been described (10) but slight modifications were introduced.

**Cells.**—Cell suspensions were made in 0.5% BSA-RPMI medium with 10 mM NaN₃ in order to prevent the redistribution of the membrane components (capping) and their further endocytosis. Incubation of the cells with antisera was routinely made for 45 min at 0°C and cells were washed at 0°C.

**Identification of TL, θ-C3H, and θ-AKR Antigens in the Thymus.**—An indirect method of fluorescence was used, i.e. after reaction of the thymus cells with the mouse alloantisera or the respective normal serum (control), the thymocyte membrane-bound mouse antibodies were detected by reaction with a second layer of FITC or TRITC Ra MγG.

The specificity of our various sera was first checked on appropriate cells. The AKR anti-θ-C3H at a final dilution of 1/10 did not stain adult AKR thymus cells; the C3H anti-θ-AKR at a final dilution of 1/100 did not stain adult C3H or BALB/c cells; and the anti-TL at a final dilution of 1/100 did not stain A-TL−, C57BL/6 (TL−), or AKR (TL−) thymus cells.

After titration of these sera on their respective thymus cells to find the highest dilution giving an optimal detection by fluorescence, we routinely used the following final dilutions for cell staining: AKR anti-θ-C3H, 1/80 for BALB/c thymus cells; C3H anti-θ-AKR, 1/100 for AKR thymus cells; and A anti-TL, 1/100 for BALB/c thymus cells. FITC or TRITC Ra MγG were used at the final concentration of 250 μg/25×10⁶ cells per ml medium. In some cases, θ on thymus cells was also identified by TRITC anti-θ-C3H, or TRITC anti-θ-AKR in the conditions described hereafter for spleen and lymph nodes.

**Identification of θ-Antigen in the Spleen and Lymph Nodes.**—A clear identification of the θ-marker required the use of directly fluorescent anti-θ antibodies, as, when the sandwich method is used for detection, the fluorescent Ra MγG would also bind to all cells with membrane immunoglobulins.

In the experiments to be reported here, we have used TRITC C3H Ig anti-θ-AKR and TRITC AKR Ig anti-θ-C3H (electrophoretic fractions “−4,” 50 μg/25×10⁶ cells per ml). It was of course checked that TRITC C3H Ig anti-θ-AKR did not bind to BALB/c thymus or lymph node cells and that TRITC AKR Ig anti-θ-C3H did not bind to AKR thymus and lymph node cells. Moreover, as checked by Roelants et al. (12), the activity of TRITC AKR Ig anti-θ-C3H was fully adsorbed out by C3H brain.

**Identification of Membrane-Bound Immunoglobulins.**—Membrane-bound immunoglobulins were detected by the direct reaction of the cells with TRITC or FITC Ra MγG. As control samples for TL and θ were also treated with normal mouse sera followed by fluorescent Ra MγG, all cells with membrane-bound immunoglobulin passively adsorbed during the in vitro incubation could also be detected.

**Microscopy.**—The cells were observed in suspension by the usual fluorescence microscopy, i.e. by looking at the cells alternatively with visible light for cell location and total number of
cells, and with specific illumination for detection of membrane fluorescence and estimation of fluorescence brightness. Cells were recorded as negative or positive. At least 500 cells were counted for each sample. We used a Leitz orthoplan microscope equipped with an Osram HBO-200 mercury lamp and an Opak-Fluor vertical illuminator (E. Leitz, Wetzlar, Germany).

In order to keep cells in a good state for counting and in order to get clean photographs, cells from each sample were also smeared on microscope slides and fixed for 5 min in absolute ethanol at room temperature. This was necessary as cells keep moving in suspension. The slides were then mounted with buffered glycerin. The photographs presented were recorded on black and white film (Kodak TRI-X-PAN 23DIIN). Exposure times were 15 s for fluorescence and 5 s for visible light (lens combination: 63 X 1.25 X 10).

Animals.—The mouse strains used were: AKR/J, BALB/c AnNicer, and a BALB/c-nu stock made by backcrossing the nude mutant to BALB/c. For thymus grafting a whole neonatal thymus or a single lobe was implanted under the shoulder skin of a nude recipient at least 6 wk old.

Spleen and bone marrow cell suspensions were made as described (13) and injected intravenously. Irradiated mice were exposed to 800 R delivered at a rate of 100 R/min by an X-ray machine operating at 300 kV and 10 mA with 2.7 mm Cu filter. Nudes that were sacrificed were checked for the absence of thymus.

RESULTS

The scope of these experiments was limited by two factors: (a) nude mice with a known genetic background are still exceptional animals and (b) the fluorochrome-conjugated mouse alloantibodies that became available only during the experiment are still extremely precious. We were therefore obliged to select which of the many possible experiments were the most immediately interesting.

In the first experiments, X-irradiated AKR mice were injected with spleen or bone marrow from BALB/c nude mice. When we looked at the lymphoid organs of those chimeras 27 days later, it appeared that both groups of mice contained some 10% of cells in the thymus, which expressed TL, and 10-20% of cells in the spleen expressing $\theta$-C3H. However, as judged by fluorescence brightness, the amount of $\theta$ expressed by these last cells was very low. Moreover, our inability to detect $\theta$-C3H on a significant proportion of cells in the thymus of these animals led us to the conclusion that this type of chimera was not a very suitable system for demonstrating T-cell precursors in the nude mice. These experiments were therefore abandoned and we concentrated our efforts on the other type of chimera studied in parallel, which gave very promising results. When BALB/c nude mice were grafted with neonatal AKR thymus ($\theta$-AKR, TL$^-$), it was evident that the thymus was repopulated by host-derived thymus cells (having $\theta$-C3H and TL) within 40-50 days. We then grafted all the available BALB/c nu/nu with neonatal AKR thymuses to look at the repopulation pattern and functional activity of these T cells. Chimeras were killed at regular intervals, and the thymus, spleen, and/or lymph nodes were assayed by immunofluorescence for the presence of TL, $\theta$-AKR, $\theta$-C3H, and membrane Ig markers. The results obtained so far are summarized in Table I and examples of $\theta$-AKR-, $\theta$-C3H-, and TL-positive cells are shown in Fig. 1.
| Time after grafting | Thymus | | Spleen | | Lymph node |
|---------------------|--------|--------|--------|--------|--------|
|                     | Ig     | Tl     | T-CHI direct* | T-CHI indirect* | Ig     | T-CHI direct | Ig     | T-CHI direct | Ig     | T-CHI direct |
| Normal AKR          | <1     | <1     | 99-100 | 99-100 | 30-35 | 0         | 30-40 |
| Normal BALB/c       | <1     | 55-80  | 99-100 | <1     | 40-55 | 50-70     | 35-55 | 52-73    | 0      |
| BALB/c-nude         |        | —      | —      | —      | 60-90 | 0         | 0      | 85-95    | 0      |
| BALB/c-nude grafted with AKR neonatal thymus |        |        |        |        |       |           |       |
| 3                   | <1     | 3      | 2      | 96     | 93    | 63.5      | 94    | 0    |
| 7                   | 4 (86) | 2 (81) | 3 (68) | 96.5   | 90    | 87        | 94    | 0    |
| 12                  |        |        |        |        |       |           |       |
| 16                  | <1     | 67.7   | 60.8   | 38.8   | 94.4  | 2.9       | 1.6 |
| 22                  | 2      | 45.6   | 28.1   | 73     | 88    | 3         | 20   |
| 26                  | <1     | 89     | 96     | 1.5    | 69    | 14.0      | 7.9  | 3.5   | 1.3 |
| 30                  | <1     | 85     | 100    | 0      | 95    | 6         | 0    |
| 37                  | <1     | 53     | 97     | 0      |       |           | 81.3 | 10.3  | 5.8 |
| 45                  | <1     | 65     | 98.8   | 99     | 0      | 42.2      |       |
| 53                  | <1     | 87.5   | 99     | 100    | 0      | 59        | 52   | 56    | 54 |

* See text for details.
† At 16 days a pool of six mice was used and at 30 days a pool of two mice.
The essential fact is that $\theta$-C3H- and TL-positive cells that can only be of host origin appear in the thymus grafts within 2-3 wk and the repopulation is completed by 4 wk. $\theta$-AKR cells that can only be derived from the thymus graft appear in lymph nodes from the grafted nude, and they are later replaced by $\theta$-C3H cells of host origin.

However, there are a few minor points that make the demonstration less clear than we had hoped. Firstly, there are the individual variations from experiment to experiment, e.g., the pool of day 16 mice show faster repopulation than the day 22 mouse. There is not enough information available yet to make any further discussion of this point worthwhile, but it shows that the repopulation of the thymus, once it has started, is rapid.

Another point is that, although soon after grafting the thymus cells resembled normal AKR cells, insofar as they did not bind normal mouse Ig, anti-TL, or anti-$\theta$-C3H, at 7 days many (86%) thymus cells bound, in vitro, small amounts of Ig from normal mouse serum, which could be detected by further incubation with TRITC $\alpha$ MγG. Of these cells only 4% exhibited membrane Ig by direct staining with anti-Ig. Only a few blast cells show strong binding of anti-$\theta$-C3H or anti-TL plus TRITC $\alpha$ MγG, but these could be B cells in the thymus. Anti-$\theta$-AKR was clearly bound by the majority of lymphocytes. We do not know whether the abnormal binding of Ig to the thymus cells has a particular biological significance or not. It was also strange that at the next stage (12 days) the thymus was so reduced in size that we could only obtain enough cells to test the $\theta$-AKR marker.

A further curious point is that during the repopulation of the thymus, cells showing detectable TL appear to be more frequent than the cells having detectable $\theta$-C3H. If this is real, it could indicate that thymus cells from AKR (TL-) are able to express TL, i.e. the AKR cells are capable of expressing TL and the AKR thymus epithelium is capable of supporting the expression of TL, but some other factor, probably a cell type, is lacking in AKR mice. However, it is not excluded in these experiments that some $\theta$-AKR cells show some TL on their membrane because TL shed from $\theta$-C3H cells sticks nonspecifically to $\theta$-AKR cells, and even the possibility that TL is a cytophilic product secreted by a few cells still remains. Specific experiments will be required to elucidate this question.

It has been routinely observed with lymph node lymphocytes from BALB/c mice (but not with lymphocytes from CBA or AKR) that the percentages of cells with demonstrable $\theta$ and with demonstrable Ig added up to slightly more

Fig. 1. Photographs of cells taken from the grafted thymus: A, 3 days; B, 16 days; and C, 30 days after grafting on the nude. Assay for detection of cells having: I, $\theta$-AKR; 2, $\theta$-C3H; and 3, TL. a, fluorescence; and b, visible light. While thymus cells taken soon after grafting only show $\theta$-AKR marker, cells with $\theta$-C3H and TL markers appear later in the thymus, which is completely repopulated by this last cell type 1 mo after grafting. Note that many of the $\theta$-C3H- and TL-positive lymphocytes, 16 days after grafting, are large lymphocytes while the $\theta$-AKR cells are small lymphocytes. X 1,500. (Fig. 1 appears on pages 1050 and 1051.)
Fig. 1
than 100%. This also occurred with these chimeras (day 22, day 26, day 53 mice). By double labeling studies (fluorescein-conjugated anti-Ig reagents and rhodamine-conjugated anti-θ), it is possible to show that a fraction of the lymphocyte population has both θ and Ig markers (F. Loot, unpublished data). Although this does not affect these studies, it shows the existence of T cells with demonstrable Ig; however, it does not show that both θ and Ig are actually produced by the cell.

A last point of interest is that at the time the grafted thymus is being repopulated, namely days 16 and 22, many blast cells (lymphoblasts, large lymphocytes) having the membrane characteristics of the host, appeared in the grafted thymus. These are shown in Table II, where we tentatively distinguish

| Group of mice | Group of cells | Cells within the group having the marker |
|---------------|---------------|-----------------------------------------|
|               |               | θ-AKR | θ-C3H | TL   |
| Day 16*       | Small lymphocytes† | 53   | 49    | 58   |
|               | Large lymphocytes‡ | 2    | 91    | 96   |
|               | Whole cell population | 38.8 | 60.8  | 67.7 |
| Day 22*       | Small lymphocytes† | 79   | 22    | 31   |
|               | Large lymphocytes‡ | 23   | 83    | 80   |
|               | Whole cell population | 73.0 | 28.1  | 45.6 |

* When the cells were classified into two categories, depending on their size, the pool of day 16 mouse thymuses was found to contain 26.8% large and medium lymphocytes and 73.2% small lymphocytes, while the day 22 thymus had 14.9% large and medium lymphocytes and 85.1% small lymphocytes.
† Small lymphocytes only.
‡ Large lymphocytes only (diameter two to three times larger than diameter of small lymphocytes) medium cells excluded.

Our results clearly demonstrate that nude mice have stem cells that are readily able to differentiate into T lymphocytes in the presence of a thymic epithelium. This has already been suggested by other authors (4, 6). In this work, the T lymphocytes were identified by use of T-lymphocyte membrane markers in chimeric nudes of appropriate strain combination. Thus, when a thymus from a neonatal AKR mouse is grafted to a nude mouse having a
BALB/c genetic background, two clear-cut T-cell membrane markers can be used: TL and \( \theta \). \( \theta \)-AKR is the Thy-1-determined antigen expressed on the membrane of T lymphocytes in a few mouse strains, including AKR, while \( \theta \)-C3H is the Thy-1-determined antigen expressed on most mouse strains, including BALB/c (7).

Similarly, the majority of BALB/c thymocytes express the TL2 antigen, while no TL antigen at all is expressed on the surface of AKR thymocytes (9). Using these two membrane antigen markers, thousands of cells could be examined by fluorescence microscopy for each thymus-grafted mouse.

While complete kinetics are not yet established, it is quite clear that lymphocytes of host origin, as identified by the presence on their membrane of TL and \( \theta \)-C3H, start to repopulate the allogeneic grafted thymus within 2 wk of grafting. At that same time, T cells of thymus donor origin can also be found in the lymph nodes of the grafted nude. The repopulation of the allogeneic grafted thymus by T cells of host origin is complete within 4 wk. At 7 wk the AKR thymus-grafted BALB/c-nudes are indistinguishable from BALB/c with regard to proportions of cells expressing TL in the thymus and of cells expressing \( \theta \)-C3H in the thymus, spleen, and lymph nodes. The changeover in the thymus seems to take place rapidly so that there is considerable individual variation between 2 and 3 wk.

Thus, precursor cells for T lymphocytes do actually exist in nu/nu mice. When an allogeneic thymus is grafted they are able to repopulate it and to pursue their differentiation; thymus-specific antigen (TL) and the T cell-specific \( \theta \)-antigen become detectable on their membranes. The results of Matsunaga and co-workers (manuscript in preparation) are in agreement with these findings. In a CBA/H T6T6 ↔ nu/nu allophenic mouse, thymus cells with the H-2 type of each parent were found although it seems probable that the thymus epithelium would have been contributed by the normal partner.

Later the thymus cells lose their TL when they leave the thymus and migrate to other lymphoid organs. In the meantime, T cells from the grafted thymus have also left the thymus and migrated to other lymphoid organs. Complete kinetic studies on those migrations will be done when more labeled anti-\( \theta \)-immunoglobulins and more nu/nu from the BALB/c backcross stock are available. Such studies might tell us how many precursors are in the nude, and how they mature. However, our studies already raise a series of important questions, relevant to problems of cell differentiation.

First, how do precursor cells reach the thymus graft? Is there a chemotactic factor or is it just chance that they encounter the thymus? If the latter is true, they would have to be swimming around everywhere in the mouse, as they reached the thymus even when it was located in a very unusual place, i.e., under the skin on the shoulder of the mouse. In any case, since the precursors cannot be detected by fluorescence microscopy, either there are very few or they express very little \( \theta \). Actually, in the spleen of nude mice, there are numbers of blast cells without easily demonstrable sur-
face immunoglobulins that seem to bind TRITC anti-\(\theta\)-C3H, but the membrane fluorescence is so faint that we can hardly detect it. Isotope-labeled anti-\(\theta\) should be used to elucidate this.

Secondly, as far as our preliminary results are concerned, it seems that the differentiation of the T precursors does not follow exactly the same pathway as in normal mice during embryogenesis. Indeed, as soon as we could find cells with TL and \(\theta\)-C3H in the thymus, small lymphocytes similar to normal adult thymus cells were already present. In contrast, cells from embryonic thymus of normal BALB/c mice, taken 13.5-14.5 days after fertilization, were only very large blast cells expressing more \(\theta\) and TL than adult thymocytes (Loor, F., and J. R. Little, data unpublished). So far we have not found such cells in the grafted thymus; where blast cells were found they were less fluorescent for \(\theta\) and TL than blasts from embryonic thymus.

This emphasizes the unusual conditions in which the host-derived T cells mature and the possible consequences of that abnormal differentiation pathway: (a) They differentiate in an allogeneic thymus and the question arises whether they will be tolerant to other tissue grafts from the donor of the thymus graft, and if not, whether they will also reject the thymus epithelium. (b) They differentiate at a very abnormal time of the animal's life-span, at a time when many fetus-specific antigens that would normally be expressed during embryogenesis no longer exist.

It is critical to determine whether the host-derived T cells display an altered panel of reactivities. It will first be necessary to demonstrate whether the bulk of host-derived T cells are physiologically active, i.e., if they can be stimulated by mitogens or if they can react against foreign antigens (cooperation with B cells in an antibody response, rejection of third-party skin grafts).

Further, it will be very interesting to see if host-derived T cells will be able to recognize host components as self, and thymus graft donor components as not-self. We are also curious to know what the reaction of these T cells will be to syngeneic embryonic cells. This should provide a useful contribution to our understanding of natural and acquired tolerance.

SUMMARY

When the thymus from an AKR mouse (TL\(^{-}\), \(\theta\)-AKR) is grafted to a BALB/c-nu/nu mouse (TL2, \(\theta\)-C3H), the grafted thymus is rapidly repopulated by host lymphocytes, i.e., lymphocytes having the TL2 and \(\theta\)-C3H T-lymphocyte membrane antigen markers. \(\theta\)-C3H lymphocytes also appear rapidly in the spleen and lymph nodes. After a few weeks, BALB/c nude mice grafted with AKR thymus and normal BALB/c mice could not be distinguished on the basis of the number of TL-positive thymocytes or \(\theta\)-C3H-positive lymphocytes in thymus, spleen, or lymph nodes.

These experiments give a definitive proof of the existence of precursor cells for the T compartment of the lymphoid system in the nude mouse. They strongly suggest the involvement of host-derived T cells in the recovery of some T-cell functions by nude mice grafted with allogeneic thymuses.

We wish to thank Doctors J. R. Little and G. Roelants for the gift of some of the antisera used and Miss Lena-Britt Haegg and Miss Dorothy Mauce for invaluable technical assistance.
One of us (F. L.) is Chargé de Recherches from the Belgian National Funds for Scientific Research (honorary title).

REFERENCES

1. Pantelouris, E. M. 1968. Absence of thymus in a mouse mutant. *Nature (Lond.).* 217:370.
2. De Sousa, M. A. B., D. M. V. Parrott, and E. M. Pantelouris. 1969. The lymphoid tissues in mice with congenital aplasia of the thymus. *Clin. Exp. Immunol. 4:*644.
3. Wortis, H. H. 1971. Immunological responses of “nude” mice. *Clin. Exp. Immunol.* 8:305.
4. Wortis, H. H., S. Nehlsen, and J. J. Owen. 1971. Abnormal development of the thymus in “nude” mice. *J. Exp. Med.* 134:681.
5. Pantelouris, E. M. 1971. Observations on the immunobiology of “nude” mice. *Immunology.* 20:247.
6. Pritchard, H., and H. S. Micklem. 1973. Haematopoietic stem cells and progenitors of functional T-lymphocytes in the bone marrow of “nude” mice. *Clin. Exp. Immunol.* 14:597.
7. Reif, A. E., and J. M. Allen. 1966. Mouse thymic iso-antigens. *Nature (Lond.).* 209:521.
8. Raff, M. C. 1971. Surface antigenic markers for distinguishing T and B lymphocytes in mice. *Transplant. Rev.* 6:52.
9. Boyse, E. A., L. J. Old, and E. Stockert. 1972. The relation of linkage group IX to leukemogenesis in the mouse. In RNA Viruses and Host Genome in Oncogenesis. P. Emmelot and P. Bentvelzen, editors. North Holland Publishing Co., Amsterdam. 171.
10. Loor, F., L. Forni, and B. Pernis. 1972. The dynamic state of the lymphocyte membrane. Factors affecting the distribution and turnover of surface immunoglobulins. *Eur. J. Immunol.* 2:203.
11. Raff, M. C. 1969. Theta isoantigen as a marker of thymus derived lymphocytes in mice. *Nature (Lond.).* 224:378.
12. Roelants, G. E., L. Forni, and B. Pernis. 1973. Blocking and redistribution (“capping”) of antigen receptors on T and B lymphocytes by anti-immunoglobulin antibody. *J. Exp. Med.* 135:956.
13. Weiler, E., and I. J. Weiler. 1968. Unequal association of mouse allotypes with antibodies of different specificities. *J. Immunol.* 101:1044.