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IMMUNE RESPONSES IN CONGENITALLY THYMUS-LESS MICE

I. ABSENCE OF RESPONSE TO OXAZOLONE

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SUMMARY

Cell proliferation in regional lymph nodes of mice sensitized with oxazolone was measured by uptake of ^{125}IUdR. Experimental factors affecting incorporation of IUdR into lymph nodes are described. The delayed hypersensitivity response was assessed by measuring the increase in ear thickness after subsequent challenge with oxazolone. Unlike normal mice, thymus-less 'nude' mice showed no detectable response to oxazolone. Implantation of an allogeneic neonatal thymus, either subcutaneously or under the kidney capsule, adoptively conferred on 'nude' mice the ability to mount a lymphoproliferative response.

INTRODUCTION

Flanagan (1966) described a recessive gene, nude (*nu*), which was associated with hairlessness in homozygotes. Pantelouris (1968) recorded that nude mice lacked a visible thymus. More recently, Pantelouris & Hair (1970) showed the thymic anlage present at 14 days of foetal life to be retarded in development. No trace of lymphoid cells was found in the thymus at this stage, or at 1 to 2 days after birth.

Nude mice die within 3–5 months of age. Death is associated with a wasting syndrome similar to that observed in neonatally thymectomized mice (Parrott, 1962). The blood leucocyte count is low (Pantelouris, 1968). The paracortical areas of the lymph nodes and spleen are deficient in lymphocytes (de Sousa, Parrott & Pantelouris, 1969), and in cells bearing the theta antigen (Raff & Wortis, 1970).

Nude mice have a severe impairment of immunological capacity. Although they can mount a limited response to sheep erythrocytes and to T4 bacteriophage (Wortis, 1971; Pantelouris 1971; Kindred, 1971), a human malignant tumour (Rygaard & Poulsen, 1969) and skin allografts and xenografts are accepted indefinitely (Rygaard, 1969; Wortis, 1971; Pantelouris, 1971; Pennycuik, 1971).

The present report describes quantitative studies on the cellular development and expression of contact sensitivity in *nu/nu* and normal control mice.

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MATERIALS AND METHODS

Mice

The recessive gene nude *nu* is found in the VII linkage group and is associated with two dominant marker genes, rex (*Re*) and trembler (*Tr*) (Flanagan, 1966). As the majority of nude mice are of low fertility, mice heterozygous for the nude gene are mated to produce the homozygous nude offspring. The colony was derived from four pairs of heterozygotes which were the gift of Professor D. S. Falconer (Dept. of Genetics, University of Edinburgh), and has subsequently been maintained in a closed but not inbred group. Thymusless nude (*nu/nu*) mice were studied in comparison with littermate *Re/+* controls, which possess a grossly normal thymus and are assumed to be in most cases heterozygous for the *nu* gene. This assumption is based on a crossover frequency of 13% between *Re* and *nu*. The mice are referred to in the text as *nu/nu* and *nu/+* respectively. Animals of both sexes were studied at 6–14 weeks of age. Male mice of the inbred strain CBA/H aged 10–15 weeks, were also used in some preliminary experiments. Neonatal CBA/H-*T6T6* mice were used as donors of thymus grafts.

Sensitization

Mice were sensitized to oxazolone (4-ethoxymethylene-2-phenyl oxazolone, BDH Chemicals Ltd.). 10 mg of oxazolone dissolved in 0·1 ml absolute ethanol at 60°C was applied to an area of skin approximately 2 cm in diameter, on the abdomen or left side of the thorax. Control animals received 0·1 ml of absolute ethanol only.

Challenge

Expression of contact hypersensitivity to oxazolone was measured by the method of Asherson & Ptak (1968). Mice were anaesthetized with ether and the thickness of each ear was measured with a dial gauge (Thomas Mercer Ltd.). Both sides of both ears were then smeared with 8 μl 2% oxazolone in olive oil, a total of 32 μl per animal. The thickness of the ears was measured again 24 hr, and sometimes also 6 and 48 hr, after challenge. The change in ear thickness after challenge in previously sensitized mice was compared with the increase in mice which received the challenge exposure only. Ear thickness was expressed in units of 10^{-3} cm.

^{125}*Iododeoxyuridine incorporation into DNA*

Cell proliferation in the lymph nodes draining an area of skin painted with oxazolone was estimated by measuring the incorporation of radiolabelled IUdR (5-iodo-2'-deoxyuridine-I^{125}, specific activity 4–7 mCi/mg, the Radiochemical Centre, Amersham), after the method of Hughes *et al.* (1964). Mice received an intraperitoneal injection of 5×10^{-8} moles fluorodeoxyuridine (FUdR) in 0·2 ml sterile distilled water, followed after 10 min by 1 μCi^{125} IUdR by the same route, and were killed 2 hr later. These standard conditions of dose and timing were established on the basis of preliminary experiments described below.

The lymph nodes draining the area treated with oxazolone (left brachial and left axillary) were excised, weighed and fixed in neutral formalin for 24 hr. Precursor not incorporated into DNA was washed from the lymph nodes with several changes of 70% ethanol until the wash was free of activity (Bryant & Cole, 1967; Elkins, 1970). Individual nodes were

counted for 2 min in a Packard Autogamma crystal scintillation spectrometer. The activity of the IUdR injected to each animal was determined and was compared with an arbitrary standard count of 1,250,000 cpm. To render the results of different experiments comparable, the activities of the individual lymph nodes were converted to this standard. No significant differences emerged in the responses of brachial and axillary nodes and the counts were therefore pooled.

Thymus grafting

A single lobe from a neonatal CBA/H-*T6T6* thymus was grafted either under the left kidney capsule, or subcutaneously in the axillary region to nude mice aged 29–58 days.

Statistics

The radioactivity counts and the lymph node weights were log-transformed to bring them into conformity with a normal distribution. Means and variances were calculated from the transformed data. No transformation was necessary for data on ear thickness. The significance of differences between experimental groups was evaluated by Student's '*t*'-test.

RESULTS

Factors affecting incorporation of ^{125}IUdR into DNA in the lymph nodes

^{125}IUdR was rapidly taken up by cells synthesizing DNA in the lymph nodes. The following experiments show the effects of varying the conditions under which incorporation was allowed to take place. CBA/H mice treated with oxazolone 3 days before testing were used in these experiments. *Nu*/+ mice showed similar, though somewhat more variable responses.

The incorporation of labelled precursor in the lymph nodes took place rapidly (Fig. 1). Half of the final level of incorporation was reached within 10–12 min of injection and a plateau was attained within 90 min.

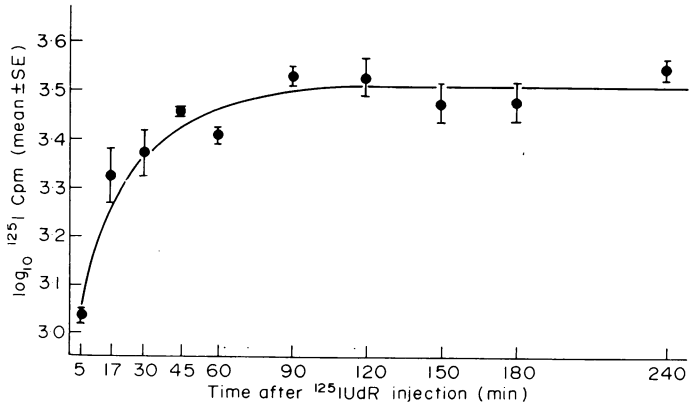

FIG. 1. Influence of time between ^{125}IUdR injection (1 μCi) and sacrifice on uptake in subcutaneous lymph nodes. Ten-week-old CBA males were sensitized with oxazolone 3 days before assay. 5×10^{-8} moles FUdR injected 10 min before ^{125}IUdR. Four mice per point.

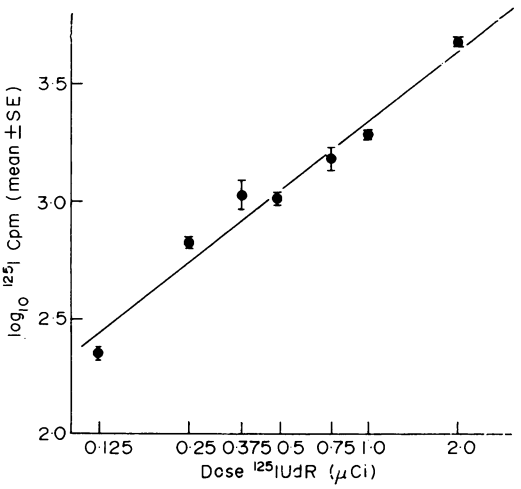

FIG. 2. Relationship of ^{125}IUdR dose to 2-hr uptake in subcutaneous lymph nodes. Fifteen-week-old CBA males were sensitized with oxazolone 3 days before assay. 5×10^{-8} moles FUdR injected 10 min before ^{125}IUdR. Four mice per point.

The amount of activity found in the lymph nodes was proportional to the amount injected to the mouse, as shown in Fig. 2.

The size of the natural thymidine pool is decreased by prior intraperitoneal injection of FUdR (Hughes *et al.*, 1964). The increase in activity in the lymph nodes when amounts of FUdR ranging from zero to 5×10^{-6} moles were injected 10 min prior to ^{125}IUdR is shown in Fig. 3. There was a twofold increase in the activity of nodes from mice receiving 5×10^{-9}, 5×10^{-8} and 5×10^{-7} moles FUdR. The lowered level of increase when 5×10^{-6} moles were injected may be due to the toxicity of the compound at this concentration (Hughes *et al.*, 1964).

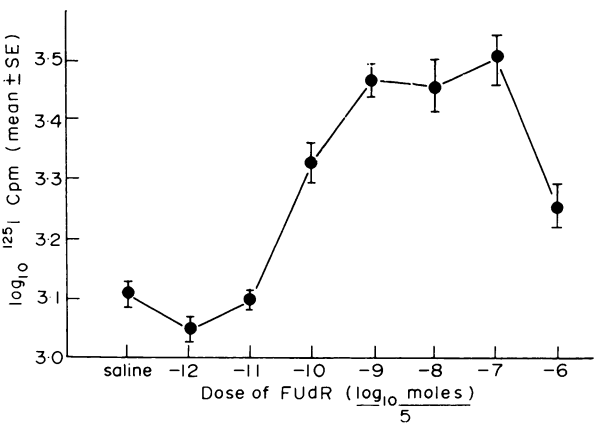

FIG. 3. Influence of FUdR, injected 10 min previously, on uptake of ^{125}IUdR (1 μCi) in subcutaneous lymph nodes. Ten-week-old CBA males were sensitized with oxazolone 3 days before assay. Four mice per point.

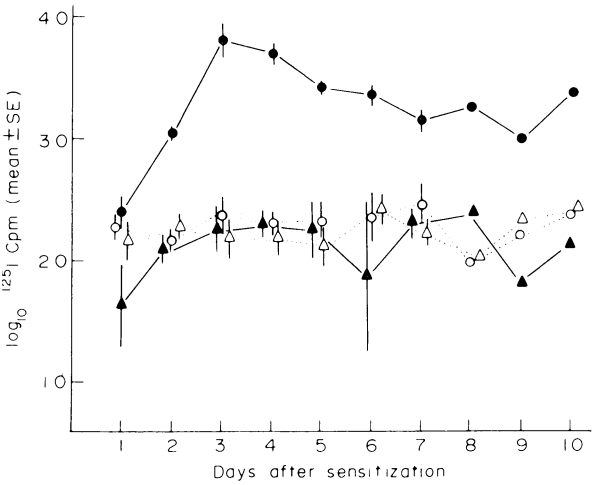

FIG. 4. ^{125}IUdR incorporation in the regional lymph nodes of oxazolone-sensitized and control mice. ● *nu*/+, sensitized; ○ *nu*/+, control. ▲ *nu*/*nu*, sensitized; △ *nu*/*nu*, control. Three mice per point on Days 1–7; one to two mice per point thereafter.

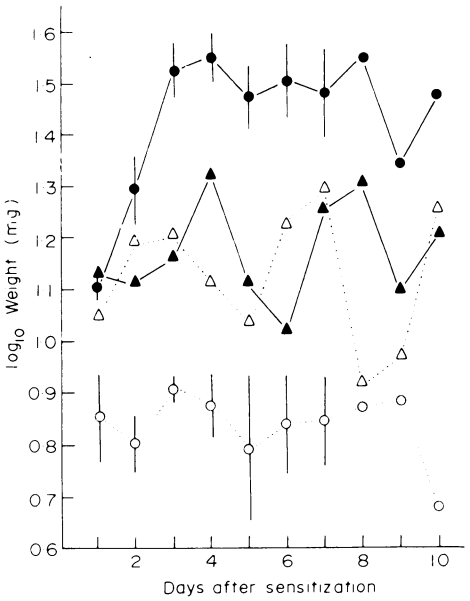

FIG. 5. Weight change in regional lymph nodes of oxazolone-sensitized and control mice. ● *nu*/+, sensitized; ○ *nu*/+, control; ▲ *nu*/*nu*, sensitized; △ *nu*/*nu*, control. Three mice per point on Days 1–7; one to two mice per point thereafter. Variances for the *nu*/*nu* groups are large (see text) and for the sake of simplicity are not indicated.

Response to sensitization with oxazolone

The cellular response of *nu/nu* mice to oxazolone was studied by daily estimations of DNA synthesis in the regional lymph nodes and was compared with the response in sensitized *nu/+* animals. Fig. 4 depicts the pooled results of three experiments with mice 6–12 weeks old. The activity in *nu/+* mice reached a peak 2-hr ^{125}IUdR incorporation of 6615 cpm on Day 3 after sensitization. Thereafter the activity declined slowly and remained above the control level, at an average 1500 cpm from Day 7 to Day 10 when the experiment was concluded. Untreated *nu/+* mice showed a low, variable uptake, ranging from 96–292 cpm. Untreated *nu/nu* mice exhibited a similarly small uptake (107–266 cpm). These presumably indicate the level of DNA synthesis in the unstimulated lymph node.

TABLE 1. Effect of neonatal CBA-*T6T6* thymus graft on cellular response in regional lymph nodes of *nu/nu* mice 3 days after sensitization with oxazolone

Group	Genotype	Thymus graft	No. of mice	Mean cpm ^{125}I ± SE*	Comparison with controls (Group 1) *P*
1	*nu/nu*	—	4	1·7916(61·9) ± 0·0458	—
2	*nu/nu*	subcutaneous	4	2·9592(910·3) ± 0·0479	< 0·001
3	*nu/nu*	kidney capsule	4	2·8543(715·0) ± 0·1315	< 0·001
4	*nu/+*	—	4	3·2680(1854·0) ± 0·0509	< 0·001

* Log^{10} (antilog of mean in parentheses)
SE = standard error of the mean.

The pattern of ^{125}IUdR incorporation in *nu/nu* mice sensitized to oxazolone could not be distinguished from that of untreated *nu/+* or *nu/nu* mice, indicating a complete absence of detectable cellular response. Values fluctuated between 42 and 251 cpm.

The weight of the lymph nodes of *nu/+* mice was raised by Day 4 after oxazolone treatment to almost five times that of non-sensitized mice. The nodes were still 3–4 times heavier at the end of the experiment. *Nu/nu* mice showed extreme variations of lymph node weight. At least some of this variation was due to gross accumulations of fluid within the nodes. Sensitization had no discernible effect. These data are represented in Fig. 5. Standard errors were very large for the *nu/nu* groups and are not represented in the figure.

A single lobe from the thymus of a CBA-*T6T6* neonate was grafted to *nu/nu* mice aged 29–58 days under the kidney capsule or subcutaneously. The mice were sensitized to oxazolone on Day 55 after grafting the thymus under the kidney capsule and on Day 40 after implantation of the thymus subcutaneously. The response to oxazolone was partially restored (Table 1). Three days after sensitization the grafted groups incorporated 11–14 times more radioactivity than did non-grafted controls.

Expression of contact hypersensitivity

The response of *nu/+* and *nu/nu* mice aged 9–14 weeks to a second contact with oxazolone was determined. The increases in ear thickness occurring when mice previously sensitized on the abdomen were challenged on the surface of the ear are detailed in Table 2.

TABLE 2. Increase in ear thickness of mice 6–48 hr after challenge with oxazolone

Genotype	Time of sensitization (days before challenge)	No. of mice	Mean increment (±SE) in ear thickness* at:				
			6 hr	24 hr	(*P*)†	48 hr	(*P*)†
nu/nu	Controls (no sensitization)	5	—	2·3±1·5		1·0±0·1	
nu/nu	4	4	—	1·4±2·5	N.S.	—	
nu/nu	7	5	1·9±0·6	2·5±0·8	N.S.	1·5±1·0	N.S.
nu/nu	12	3	—	4·6±0·2	N.S.	1·2±1·2	N.S.
nu/+	Controls (no sensitization)	5	—	11·2±1·8		5·1±1·2	
nu/+	4	4	—	26·2±2·4	< 0·001		
nu/+	7	5	15·5±2·5	22·5±1·3	< 0·001	11·7±2·0	< 0·05
nu/+	12	3	—	21·0±3·9	< 0·05	15·7±2·0	< 0·01

* Units of 10^{-3} cm.
† Significance of difference from controls (Student's '*t*'-test).
N.S. = not significant.

The mice were challenged 4, 7 and 12 days after sensitization. Whereas the ears of *nu/+* mice showed significant thickening compared with non-sensitized controls, no such effect was observed in *nu/nu* mice. Indeed, a decrease in ear thickness after challenge was observed in a number of *nu/nu* mice. In addition to mice in the groups which showed a mean decrease (Table 2), two animals from the group challenged at Day 4, and two from Day 12, measured at 48 hr also showed reduced ear thickness.

Sensitization on the abdomen tended, by itself, to be followed by an increase in ear thickness. This tendency was more marked in *nu/nu* than in *nu/+* mice, although in absolute terms *nu/nu* ears were thinner than *nu/+* throughout the experiment. Some thickening was also seen after 'challenge' of non-sensitized mice (Table 3).

TABLE 3. Ear thickness in *nu/nu* and *nu/+* mice treated with either a sensitizing or a challenge dose of oxazolone, or untreated

Genotype	No. of mice	Treatment	Day after treatment	Ear thickness* (mean±SE)	(*P*)†
nu/nu	5	—	—	19·4±1·7	
nu/nu	4	Sensitized	4	30·8±1·8	< 0·01
nu/nu	5	Sensitized	7	23·9±0·8	< 0·05
nu/nu	3	Sensitized	12	20·5±2·5	N.S.
nu/nu	5	Challenged	1	21·7±2·0	N.S.
nu/nu	5	Challenged	2	20·5±1·6	N.S.
nu/+	5	—	—	27·4±3·2	
nu/+	5	Sensitized	4	34·2±2·9	N.S.
nu/+	5	Sensitized	7	35·3±1·2	N.S.
nu/+	3	Sensitized	12	29·8±5·1	N.S.
nu/+	5	Challenged	1	38·6±2·8	< 0·05
nu/+	5	Challenged	2	32·4±2·2	N.S.

* Units of 10^{-3} cm.
† Significance of difference from untreated controls of same genotype (Student's '*t*'-test).
N.S. = not significant.

DISCUSSION

Incorporation of radiolabelled IUdR has been extensively used to measure DNA synthesis and hence to provide an estimate of cell proliferation both in tumours and in normal haematopoietic and other tissues (Hughes *et al.*, 1964; Bryant & Cole, 1967; Bennett *et al.*, 1968; Dethlefsen, 1971). Mäkelä & Mitchison (1965) used the technique to measure the cellular response to antigenic stimulation with bovine serum albumin. The present results show that assay conditions as described by Hughes *et al.* (1964) can be successfully applied to the study of cell proliferation in normal and stimulated lymph nodes.

The consistently low level of IUdR incorporation shown by the lymph nodes of non-stimulated *nu*/+ mice provided a satisfactory baseline against which to view the response of sensitized animals. The latter incorporated, at the peak on Day 3, some 30 times more IUdR than the controls. Some pilot studies with +/+ mice (unpublished data) gave similar values, indicating that possession of the *nu* gene in single dose does not affect the response to oxazolone. In contrast, *nu*/*nu* mice, while showing a background level of incorporation similar to that of *nu*/+ animals, gave no detectable response to oxazolone. A significant response was conferred adoptively to *nu*/*nu* mice by a graft of allogeneic neonatal thymus. It is not yet known whether this response was effected entirely by donor cells which migrated out of the thymus graft into the peripheral lymphoid tissues (Davies *et al.*, 1966) or whether the graft also enabled host precursors to mature into responsive cells.

The effectiveness of an allogeneic graft is presumably related to the known inability of *nu*/*nu* mice to reject foreign tissue grafts.

In contrast to the IUdR incorporation assay, the measurement of lymph node weights was both less sensitive and less consistent. The weight of *nu*/+ lymph nodes increased after oxazolone sensitization when compared with nodes from unsensitized controls. However, *nu*/*nu* lymph node weights varied widely and those from sensitized animals were no heavier than those from non-sensitized controls. *Nu*/*nu* lymph nodes were on average heavier than those from non-sensitized *nu*/+ mice and this additional weight was frequently associated with an accumulation of fluid in the lymph nodes of both non-sensitized and sensitized *nu*/*nu* mice.

Contact sensitivity to oxazolone, assayed by increase in ear thickness (Asherson & Ptak, 1968) could be demonstrated in *nu*/+ mice following a single painting of antigen on the abdomen 4, 7 or 12 days before challenge. In contrast, the ears of sensitized *nu*/*nu* mice did not increase in thickness at challenge and in thirteen of twenty-five mice examined, a decrease in thickness was observed. Thus, both the development and expression of contact sensitivity are absent in *nu*/*nu* mice.

After sensitization on the abdomen the ears of both *nu*/+ and *nu*/*nu* mice were observed to be thicker than in non-sensitized animals. This may have been a non-specific, physiological effect of irritation caused by the oxazolone. Some mild skin lesions and increased vascularization of the treated area were evident in *nu*/*nu* mice despite the apparent failure to develop contact sensitivity. Since increased thickness was observed after application of oxazolone to the ears of unsensitized mice, the ear swelling seen in sensitized mice before challenge may be due to accidental transfer of oxazolone from the abdomen to the ears, perhaps during grooming.

It is known that the development of delayed hypersensitivity is thymus dependent (Arnason *et al.*, 1962; de Sousa & Parrott, 1969; Parrott *et al.*, 1970). It involves early cell

proliferation in the paracortical area of lymph nodes in guinea-pigs (Oort & Turk, 1965; Turk, 1967), and mice (Parrott & de Sousa, 1966). The presence of lymphocytes in the paracortical area depends on the animal possessing an intact thymus (Parrott & de Sousa, 1966). Davies *et al.* (1969) showed early proliferation of thymus-derived cells ('T-cells' in modern parlance (Roitt *et al.*, 1969)) in the lymph node paracortex of mice sensitized with oxazolone. At a later stage, bone marrow derived 'B' cell proliferation was observed and it coincided with germinal centre formation and hyperplasia of the medullary cords. T-cell division reached a peak on Day 4 after sensitization while the peak B-cell proliferation was observed at Day 8, and was sustained at a slightly lower level for the remainder of the experiment. The results of the IUdR incorporation assay are consistent with these data. The peak incorporation at Day 3 in *nu/+* mice is presumably due to synthesis of DNA by T-cells while the continuing incorporation from Day 7–8 onwards may be associated with proliferation of B-cells. The *nu/nu* mice showed no response even in the period of B-cell proliferation although the evidence to date suggests that *nu/nu* mice have a normal B-cell population (Wortis, 1971). The B-cell response in thymectomized mice is much reduced and short-lived (Davies *et al.*, 1969). The evidence, therefore, suggests that the B-cell response to oxazolone sensitization is dependent on a previous T-cell response. The failure of sensitized *nu/nu* mice to show increased ear thickness after challenge with oxazolone confirms the view that the ability to express contact sensitivity to oxazolone is absent in thymusless mice (de Sousa & Parrott, 1969; Parrott *et al.*, 1970). It may be that thymus-derived cells infiltrate the reactive site during the early stages of hypersensitivity reactions in mice as has been shown to occur in rats (Williams & Waksman, 1969).

Several workers have reported that *nu/nu* mice give subnormal responses to SRBC (Wortis, 1971; Kindred, 1971; Pantelouris, 1971) and are unable to reject skin allografts and xenografts (Rygaard, 1969; Pantelouris, 1971; Pennycuik, 1971; Wortis, 1971). Wortis (1971) found no increase in cellularity of draining lymph nodes following injection of PHA into the footpad of *nu/nu* mice and only slight lymphocytosis after intravenous injection of *B. pertussis* organisms. Both these responses are reported to depend on an intact thymus (Dukor & Dietrich, 1967; Kalpaktsoglou *et al.*, 1969). The present data show a total failure to respond in a system well known to be T-cell dependent. Partial restoration of the response to oxazolone in thymus grafted *nu/nu* mice extends the observation that after implantation of a thymus graft or intraperitoneal injection of thymus cells, *nu/nu* mice were able to reject skin allografts (Pantelouris, 1971).

The primary cause of congenital absence of thymus in *nu/nu* mice is not known. It may be due to abnormal development of the thymic anlage in foetal life (Pantelouris & Hair, 1971) but this could itself be associated with absence or abnormal development of the extrathymic precursors of thymus lymphocytes, which have been shown to be present in yolk-sac and foetal liver (Tyan, 1968; Owen & Ritter, 1969; Moore & Metcalf, 1970) and in adult bone marrow (Micklem, Ford, Evans & Gray, 1966) of normal mice. The thymus grafting experiments reported here do not resolve the question, since graft-derived T-cells might survive long enough to produce the observed response by themselves, without the occurrence of any functional maturation of host T-cells. Experiments are in progress to investigate the presence of potential T-cell precursors in *nu/nu* mice.

Among the cases of primary immunological deficiency diseases known in man, three syndromes are described involving congenital aplasia or hypoplasia of the thymus (Hitzig, 1968). The defects observed in two of these bear some resemblance to those of *nu/nu* mice.

In Nezelof's isolated lymphopenia transmitted by an autosomal recessive gene, the thymus is hypoplastic, and the lymph nodes develop abnormally, but plasma cells are present despite the severe lymphopenia (Nezelof *et al.*, 1964; Fulginiti *et al.*, 1966). DiGeorge's syndrome (DiGeorge, 1954; Lischner *et al.*, 1967) is typified by congenital aplasia of the thymus accompanied by aplasia of the parathyroids and hypoplasia of the mandibula. The derivatives of the III and IV pharyngeal pouches are not abnormal in *nu/nu* mice (Pantelouris & Hair, 1970). The aplasia of the lymphoid system in cases of DiGeorge's syndrome is apparently limited to the thymus-dependent cell population, and implantation of a thymus gland is an effective replacement therapy (August *et al.*, 1968; Cleveland *et al.*, 1968). Owen & Ritter (1969) postulated that the primary defect in DiGeorge's syndrome was thymic, while in other thymic aplasias there was additional failure of immigrant stem cells, or of stem cell-organ rudiment interaction. Although the immunological responses of *nu/nu* mice can be partially restored by thymus grafting, this may be a transient result of the responsiveness of cells derived from the original graft lymphocytes. Further studies are needed to elucidate the precise nature of the defect and its relationship to human disease-states.

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REFERENCES

ARNASON, B.G., JANKOVIC, B.D., WAKSMAN, B.H. & WENNERSTEN C. (1962) Role of the thymus in immune reactions in rats: II. Suppressive effect of thymectomy at birth on reactions of delayed (cellular) hypersensitivity and the circulating small lymphocyte. *J. exp. Med.* **116,** 177.

ASHERSON, G.L. & PTAK, W. (1968) Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. *Immunology*, **15,** 405.

AUGUST, C.S., ROSEN, F.S., FILLER, R.M., JANEWAY, C.A., MARKOWSKI, B. & KAY, H.E.M. (1968) Implantation of a foetal thymus, restoring immunological competence in a patient with thymic aplasia (DiGeorge's sydrome). *Lancet*, **ii,** 1210.

BENNETT, M., PINKERTON, P.H., CUDKOWICZ, G. & BANNERMAN, R.M. (1968) Hemopoietic progenitor cells in marrow and spleen of mice with hereditary iron deficiency anemia. *Blood*, **32,** 908.

BRYANT, B.J. & COLE, L.J. (1967) Evidence for pluripotentiality of marrow stem cells: modification of tissue distribution of *in vivo* ^{125}I-UdR labelled transplanted marrow. *The Lymphocyte in Immunology and Haemopoiesis* (Ed. by J. M. Yoffey), p. 170. Edward Arnold.

CLEVELAND, W.W., FOGEL, B.J., BROWN, W.T. & KAY, H.E.M. (1968) Foetal thymic transplant in a case of DiGeorge's syndrome. *Lancet*, **ii,** 1211.

DAVIES, A.J.S., LEUCHARS, E., WALLIS, V. & KOLLER, P.C. (1966) The mitotic response of thymus-derived cells to antigenic stimulus. *Transplantation*, **4,** 438.

DAVIES, A.J.S., CARTER, R.L., LEUCHARS, E. & WALLIS, V. (1969) The morphology of immune reactions in normal, thymectomized and reconstituted mice. II. The response to oxazolone. *Immunology*, **17,** 111.

DE SOUSA, M.A.B. & PARROTT, D.M.V., (1969) Induction and recall in contact sensitivity. Changes in skin and draining lymph nodes of intact and thymectomized mice. *J. exp. Med.* **130,** 671.

DE SOUSA, M.A.B., PARROTT, D.M.V. & PANTELOURIS, E.M. (1969) The lymphoid tissues in mice with congenital aplasia of the thymus. *Clin. exp. Immunol.* **4,** 637.

DETHLEFSEN, L.A. (1971) An evaluation of radioiodine-labelled 5-iodo-2′-deoxyuridine as a tracer for measuring cell loss from solid tumours. *Cell Tissue Kinet.* **4,** 123.

DIGEORGE, A.M. (1965) Discussion of a paper by M. D. Cooper *et al.*, *J. Pediat.* **67,** 907.

DUKOR, P. & DIETRICH, F.M. (1967) Impairment of phytohaemagglutinin induced blastic transformation in lymph nodes from thymectomized mice. *Int. Arch. Allergy*, **32,** 521.

ELKINS, W.L. (1970) Specific and non-specific lymphoid cell proliferation in the pathogenesis of graft-versus-host reactions. *Transplantation*, **9,** 273.

FLANAGAN, S.P. (1966) 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genet. Res.* (*Camb.*) **8,** 295.

FULGINITI, V.A., HATHAWAY, W.E., PEARLMAN, D.S., BLACKBURN, W.R., REIQUAM, C.W., GITHENS, J.H., CLAMAN, H.N. & KEMPE, C.H. (1966) Dissociation of delayed-hypersensitivity and antibody synthesising capacities in man. *Lancet*, **ii,** 5.

HITZIG, W.H. (1968) Combined cellular and humoral deficiency states. *Proc. roy. Soc. Med.* **61,** 887.

HUGHES, W.L., COMMERFORD, S.L., GITLIN, D., KRUEGER, R.C., SCHULTZE, B., SHAH, V. & REILLY, P. (1964) Deoxyribonucleic acid metabolism *in vivo:* I. Cell proliferation and death as measured by incorporation and elimination of iododeoxyuridine. *Fed. Proc.* **23,** 640.

KALPAKTSOGLOU, P.K., YUNIS, E.J. & GOOD, R.A. (1969) Changes produced by pertussis antigen on the blood cells and lympho-haemopoietic tissues after early and late thymectomy or splenectomy. *Clin. exp. Immunol.* **5,** 91.

KINDRED, B. (1971) Immunological unresponsiveness of genetically thymusless nude mice. *Eur. J. Immunol.* **1,** 59.

LISCHNER, H.W., PUNNETT, H.H. & DIGEORGE, A.M. (1967). Lymphocytes in congenital absence of the thymus. *Nature* (*Lond.*), **214,** 580.

MÄKELÄ, O. & MITCHISON, N.A. (1965) The effect of dosage on the response of adoptively transferred cells. *Immunology*, **8,** 549.

MICKLEM, H.S., FORD, C.E., EVANS, E.P. & GRAY, J. (1966) Interrelationships of myeloid and lymphoid cells: studies with chromosome-marked cells transfused into lethally irradiated mice. *Proc. roy. Soc. B.* **165,** 78.

MOORE, M.A.S. & METCALF, D. (1970) Ontogeny of haemopoietic system: Yolk sac origin of *in vivo* and *in vitro* colony forming cells in the developing mouse embryo. *Brit. J. Haematol.* **18,** 279.

NEZELOF, C., JAMMET, M.L., LORTHOLARY, P., LABRUNE, B. & LAMY, H. (1964) L'hypoplasie hereditaire du thymus. *Arch. franc. Pediat.* **21,** 897.

OORT, J. & TURK, J.L. (1965) A histological and autoradiographic study of lymph nodes during the development of contact sensitivity in the guinea pig. *Brit. J. exp. Path.* **46,** 147.

OWEN, J.J.T. & RITTER, M.A. (1969) Tissue interaction in the development of thymus lymphocytes. *J. exp. Med.* **129,** 431.

PANTELOURIS, E.M. (1968). Absence of a thymus in a mouse mutant. *Nature* (*Lond.*), **217,** 370.

PANTELOURIS, E.M. (1971) Observations on the immunobiology of 'nude' mice. *Immunology*, **20,** 247.

PANTELOURIS, E.M. & HAIR, J. (1970) Thymus dysgenesis in *nude* (nunu) mice. *J. Embryol. exp. Morph.* **24,** 615.

PARROTT, D.M.V. (1962) Strain variation in mortality and runt disease in mice thymectomized at birth. *Transplantation Bull.* **29,** 102.

PARROTT, D.M.V. & DE SOUSA, M.A.B. (1966) Changes in thymus-dependent areas of lymph nodes after immunological stimulation. *Nature* (*Lond.*), **212,** 1316.

PARROTT, D.M.V., DE SOUSA, M.A.B., FACHET, J., WALLIS, V., LEUCHARS, E. & DAVIES, A.J.S. (1970) The response of normal, thymectomized and reconstituted mice in contact sensitivity. *Clin. exp. Immunol.* **7,** 387.

PENNYCUIK, P.R. (1971) Unresponsiveness of nude mice to skin allografts. *Transplantation*, **11,** 417.

RAFF, M.C. & WORTIS, H.H. (1970) Thymus dependence of θ-bearing cells in the peripheral lymphoid tissues of mice. *Immunology*, **18,** 931.

ROITT, I.M., GREAVES, M.F., TORRIGIANI, G., BROSTOFF, J. & PLAYFAIR, J.H.L. (1969) The cellular basis of immunological responses. *Lancet*, **ii,** 367.

RYGAARD, J. (1969) Immunobiology of the mouse mutant 'nude'. *Acta path. microbiol. scand.* **77,** 761.

RYGAARD, J. & POULSEN, C.O. (1969) Heterotransplantation of a human malignant tumour to Nude Mice. *Acta path. microbiol. scand.* **77,** 758.

TURK, J.L. (1967) *Delayed Hypersensitivity*, p. 142. North Holland Publishing Co., Amsterdam.

TYAN, M.L. (1968) Studies on the ontogeny of the mouse immune system I. Cell-bound immunity. *J. Immunol.* **100,** 535.

WILLIAMS, R.M. & WAKSMAN, B.H. (1969) Thymus-derived cells in the early phase of delayed tuberculin reactions. *J. Immunol.* **103,** 1435.

WORTIS, H.H. (1971) Immunological responses of 'nude' mice. *Clin. exp. Immunol.* **8,** 305.