Clin. exp. Immunol. (1973) **13**, 125–138.

IMMUNE RESPONSES IN CONGENITALLY THYMUS-LESS MICE

II. QUANTITATIVE STUDIES OF SERUM IMMUNOGLOBULINS, THE ANTIBODY RESPONSE TO SHEEP ERYTHROCYTES, AND THE EFFECT OF THYMUS ALLOGRAFTING

HELEN PRITCHARD, JOSEPHINE RIDDAWAY AND H. S. MICKLEM

Immunobiology Unit, Department of Zoology, University of Edinburgh

(*Received* 19 *July* 1972)

SUMMARY

The concentrations of immunoglobulins in the sera of congenitally thymus-less 'nude' (*nu/nu*) mice of various ages were studied by single radial diffusion and compared with those in phenotypically normal *nu/+* controls. γM was present in normal concentrations. γA and γG2a concentrations were greatly reduced. γG1 was normal at the time of weaning (28 days of age), but fell progressively thereafter until it was indetectable in most animals more than 60 days old. The primary response to 4×10^7 sheep erythrocytes was studied by haemolytic plaque and serum haemagglutination techniques. *Nu/nu* mice made subnormal quantities of 19S and little or no 7S antibody. Subcutaneous grafting of neonatal CBA/H thymus at 10–25 days of age was followed by increased survival of *nu/nu* mice and by the appearance of normal concentrations of γG2a and γG1 globulins and near-normal concentrations of γA. Intraperitoneal injection of 10^8 CBA/H thymus cells resulted in still higher concentrations of these three proteins. It is concluded that the establishment of normal serum concentrations of γA, γG2a and, especially, γG1 depends upon the presence of funtional T-lymphocytes. γM shows no such T-dependence.

INTRODUCTION

The recessive gene nude (*nu*) first described by Flanagan (1966) is associated with hairlessness and congenital aplasia of the thymus in mice (Pantelouris, 1968). An abnormal thymic rudiment is described in *nu/nu* mice which never becomes lymphoid (Pantelouris & Hair, 1970; Wortis, Nehlson & Owen, 1971). The thymus-dependent areas of the peripheral lymphoid tissues are severely depleted of lymphocytes (de Sousa, Parrott & Pantelouris,

Correspondence: Dr H. S. Micklem, Immunobiology Unit, Department of Zoology, West Mains Road, Edinburgh, EH9 3JT.

1969), and the number of lymphocytes bearing the θ-antigen is at most very low (Raff & Wortis, 1970). The immune responses of *nu/nu* mice show to an extreme degree the abnormalities well-known to be associated with neonatal thymectomy in rodents. Thus cell-mediated responses and antibody formation against various antigens are profoundly impaired (Rygaard, 1969; Wortis, 1971; Pantelouris, 1971; Kindred, 1971a; Pennycuik, 1971; Pantelouris & Flisch, 1972; Pritchard & Micklem, 1972; Reed & Jutila, 1972). The antibody response to the thymus-independent antigens *Escherichia coli* lipopolysaccharide and pneumococcal SIII polysaccharide is, however, reported to be normal (Manning, Reed & Jutila, 1972).

Studies of neonatally thymectomized rodents have not shown any very consistent and substantial abnormality in immunoglobulin levels (Humphrey, Parrott & East, 1964; Fahey, Barth & Law, 1965; Arnason, de Vaux St. Cyr & Shaffner, 1964), although low levels of γG2a and γG1 were often observed, particularly in wasting mice (Humphrey *et al.*, 1964; Fahey *et al.*, 1965), and Arnason *et al.* (1964) described reduced levels of a protein probably corresponding to γG1. Bazin & Duplan (1966), on the other hand, found raised serum immunoglobulin concentrations in some long-lived thymectomized radiation chimaeras. Benveniste, Lespinats & Salomon (1969) found that neonatally thymectomized axenic mice had higher concentrations of γG1 and γG2 globulins than did axenic controls, although they were lower than in conventionally reared animals. Neonatally thymectomized and thymectomized-irradiated mice are likely to have a certain number of thymus-processed cells (T-cells) (Raff & Wortis, 1970), and it is not known how much this residual population may influence immunoglobulin levels.

The present paper describes quantitative studies on the serum concentrations of four major classes of immunoglobulin and on antibody production to sheep erythrocytes in nude mice. The influence of thymus grafts on immunoglobulin concentrations is also described.

MATERIALS AND METHODS

Mice

Nude (*nu/nu*) mice were maintained in a closed, but not inbred colony, as previously described (Pritchard & Micklem, 1972). They were weaned at 4 weeks. Comparisons were made with age-matched mice heterozygous for the nude gene (*nu/+*). Although the possibility that the *nu* gene in single dose may confer some minor immunological abnormality, has not been excluded, *nu/+* mice are assumed to be 'normal' for the purposes of comparison.

Thymus grafting

One or two lobes from a neonatal CBA/H thymus were grafted subcutaneously in the axillary region of nude mice aged 10–25 days. Nude controls were sham-grafted. Serum samples were obtained 5 or 6 weeks later.

Thymus cells

2×10^8 thymocytes from CBA/H donors aged 3–4 weeks were injected in 0·3 ml Hanks' solution to nude mice aged 26–32 days. Nude controls received 0·3 ml Hanks' only. Serum samples were obtained 6 weeks later.

Preparation of specific antisera

The mouse immunoglobulins γM, γG_1, γG2a and γA were purified from the serum or (for γA) urine of mice bearing the myelomas MOPC 104F, RPC23, 5563, and MOPC 47a respectively. These tumours were obtained from the National Institute for Medical Research, Mill Hill, by courtesy of Dr B. A. Askonas and Dr D. W. Dresser, and maintained by serial passage. γM was purified on the basis of its euglobulin characteristics on Sephadex G-25 (Pharmacia) (Bazin, Savin & Micklem, 1968), followed by gel filtration on Sephadex G-200. γG1 and γG2 were purified by ion exchange chromatography on DEAE cellulose (DE52: Whatman) using a linear gradient of 0·01 M–0·03 M phosphate buffer at pH 8, followed by three precipitations with 45% saturated ammonium sulphate. Urine from mice carrying MOPC 47a contains half-molecules of γA, traces of albumin being the only detectable protein contaminant. The urine was dialysed against phosphate-buffered saline (pH 7·4) and concentrated by pressure ultra-filtration through a Pellicon PSED membrane (Millipore). Rabbits were immunized with 500 μg–5 mg of purified protein emulsified in Freund's complete adjuvant (Difco) plus 10^{10} killed *Bordetella pertussis* organisms (Wellcome), subcutaneously at several sites on their sides. They were bled 5 weeks later. Antibodies against L-chain determinants were removed from the antisera by passage through a column of Sepharose 4B (Pharmacia) to which was coupled one or more of the myeloma proteins not used for immunization (Porath *et al.*, 1967), or by repeated precipitation with purified myeloma protein. The specificity of each anti-serum was checked by immuno-electrophoresis and double diffusion.

Antisera were also raised in a similar fashion against normal serum immunoglobulins, by injecting *B. pertussis* organisms which had been coated with mouse anti-pertussis antibodies and washed four times; and against γG globulins purified from normal serum by DEAE cellulose chromatography. These antisera were used without absorption.

Quantification of immunoglobulins

The single radial immunodiffusion assay described by Mancini, Carbonara & Heremans (1965) was used to quantify γG1, γG2a, γA and γM globulins in serum obtained from untreated nude (*nu/nu*) and normal (*nu/+*) mice, and from *nu/nu* mice grafted with CBA thymus or injected with CBA thymus cells.

Briefly, an equal volume of 3% Special Agar-Noble (Difco) in veronal-HCl buffer at 60°C (pH 8·6 and ionic strength 0·1) was mixed with a suitable dilution of antiserum in veronal-HCl buffer at 55°C and poured into a mould of two photographic plates and a brass support. A layer of agar-antiserum measuring 8·5 × 6·2 cm and 1-mm thick, was thus formed on one of the plates. Circular wells of 2 mm diameter were punched out of the gel, and the agar removed by suction. 2·5 μl of antigen, either test serum, standard serum or dilutions of these, was placed in each well. The plates were maintained for 14 days at 37°C under a layer of paraffin oil. The oil was removed by rinsing with petroleum ether; the plates were washed with several changes of veronal-saline (1·5% NaCl with 4% v/v veronal buffer), dried and stained consecutively with 0·1% ponceau red and 0·1% nigrosin in 0·1 M acetate buffer (pH 5).

The diameters of the ring-shaped precipitates formed round the antigen wells were measured with a precision viewer (Hyland), and the areas within the rings were calculated.

The area of the precipitate is directly proportional to the quantity of the immunoglobulin

class in the sample under test. The quantity of each immunoglobulin class in each serum sample was expressed as per cent of the quantity found in a standard serum pool obtained from 3–6-month-old normal outbred mice. Standard lines obtained from assaying four dilutions of this serum pool on each plate were used in calculating the proportion of the standard immunoglobulin level found in all test sera on that plate.

In addition to standard and experimental sera, a sample of a single control serum was assayed on every plate. Analysis of the results from this repeated assay showed that 95% of the individual observations fell within 3% of the mean.

The lower limits of the sensitivity of the assay expressed as per cent of the level in the standard pool were approximately 1% for γG1; 3% for γG2a; 5% for γA and 9% for γM.

The results were log-transformed for statistical comparison by Student's *t*-test.

Antibody response

The 19S and 7S antibody responses of *nu/nu* and *nu/+* female mice aged 9–14 weeks, to 4×10^7 sheep red blood cells (SRBC) injected intraperitoneally were determined by an adaptation (Wortis, Taylor & Dresser, 1966; Dresser & Wortis, 1967) of the haemolytic plaque assay of Jerne, Nordin & Henry, 1963. Agarose (L'Industrie Biologique Française, Gennevilliers, France) was used for both top and bottom layers. A phosphate-buffered balanced salt solution (Mishell & Dutton, 1967) was employed in preference to a bicarbonate-containing medium. A rabbit antiserum directed against components of all major mouse immunoglobulin classes was used at a dilution of 1/1000 to develop 7S plaque-forming cells, after initial tests to establish its inhibition and development constants.

Circulating 19S (2-mercaptoethanol-sensitive) and 7S (2-mercaptoethanol-resistant) antibody was measured by a haemagglutination assay. 0·05 ml of each serum sample was diluted serially in gelatin-PBS (phosphate-buffered saline, pH 7·2, containing 1% gelatin), starting at a dilution of 1 in 2, in Linbro HA trays. Two dilution series were made for each serum sample. 0·05 ml of 0·75% SRBC was added to one series and the trays were covered with Microtitre plate sealer (Cooke Engineering Co.) and incubated at 37°C for 1 hr, and overnight at 4°C. The second series received 0·05 ml of 0·2 M 2-mercaptoethanol, in addition to SRBC (Claflin, Smithies & Meyer, 1966) and were covered and incubated in the same way. The titre was read as the last well showing macroscopic agglutination. The supernatant was then removed without disturbing the pellet, and 0·1 ml of 1/1000 dilution of a rabbit antiserum to normal mouse γG-globulins was added to all wells of both series. The trays were re-covered and again incubated for 1 hr at 37°C and overnight at 4°C. The titre was again read as the last well showing macroscopic agglutination.

RESULTS

Immunoglobulin concentrations in untreated mice

The concentration of γA, γG2a, γG1 and γM immunoglobulins in the sera of fifteen untreated male and female *nu/nu* mice, aged 52–64 days, and in fifteen *nu/+* controls of similar age are shown in Table 1. The levels of γA, γG2a and γG1 in *nu/nu* mice were significantly lower than those of the controls ($P < 0{\cdot}001$), while γM was present in normal amounts. The most marked reduction was in γG1, of which *nu/nu* had less than 4% of the normal quantity.

In neonatally thymectomized mice, Humphrey *et al.* (1964) noted a delay in production of immunoglobulins following the hypogammaglobulinaemia seen in all young mice. One

TABLE 1. Serum immunoglobulin concentrations in fifteen *nu/nu* and fifteen *nu/+* male and female mice aged 52–64 days

Genotype	Mean percentage of standard serum			
	γA	γG2a	γG1	γM
nu/nu	7·5	19·1	2·5	107·0
nu/+	59·7	88·8	71·2	90·2
t	5·28	7·17	13·57	0·88
d.f.	28	28	25	25
P	<0·001	<0·001	<0·001	>0·2

explanation of the above results could therefore have been that the delay was more prolonged in *nu/nu* mice. Accordingly, sera from forty-one *nu/nu* of various ages and 34 age-matched controls were assayed. The results are shown in Figs 1–4. Eight of the *nu/nu* mice showed elevated γM levels. The concentrations of γA and γG2a were consistently below those in the controls at all ages. γA was undetectable in more than half the *nu/nu* mice up to 2 months of age, but tended to increase slightly. Since no change occurred when the *nu/nu* animals were isolated from their normal littermates, the increase in γA cannot be attributed to coprophagy followed by absorption of γA from the gut. At the earliest ages tested, the amount of γG1 was normal, but while the level rose in control mice during the second month

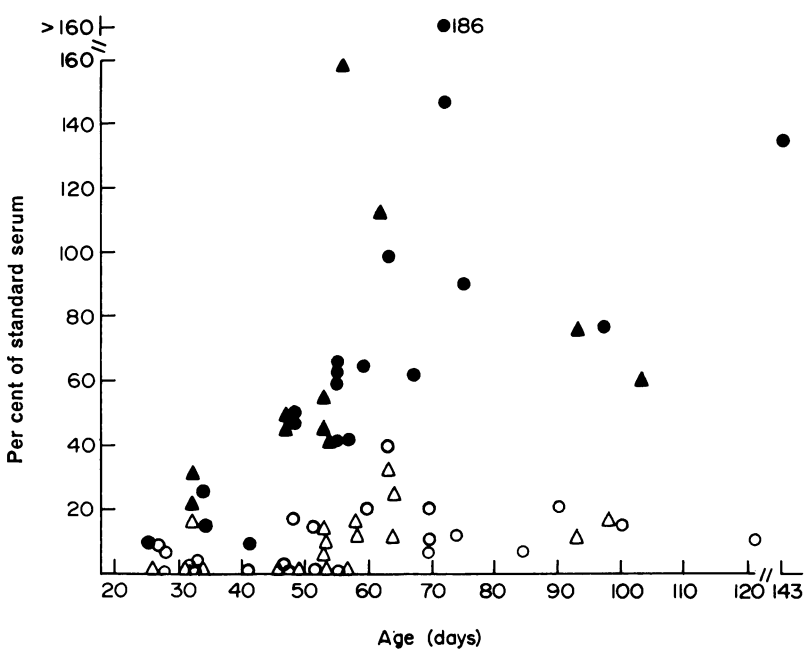

FIG. 1. Relationship of serum γA concentration to age in *nu/nu* and *nu/+* mice. ○, *nu/nu* male; △, *nu/nu* female; ●, *nu/+* male; ▲, *nu/+* female.

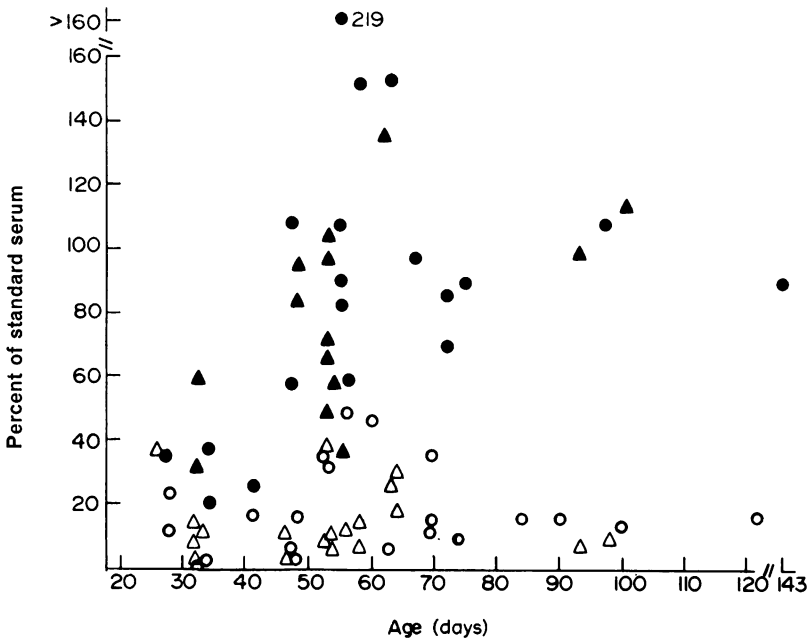

FIG. 2. Relationship of serum γG2a concentration to age in *nu/nu* and *nu/+* mice. ○, *nu/nu* male; △, *nu/nu* female; ●, *nu/+* male; ▲, *nu/+* female.

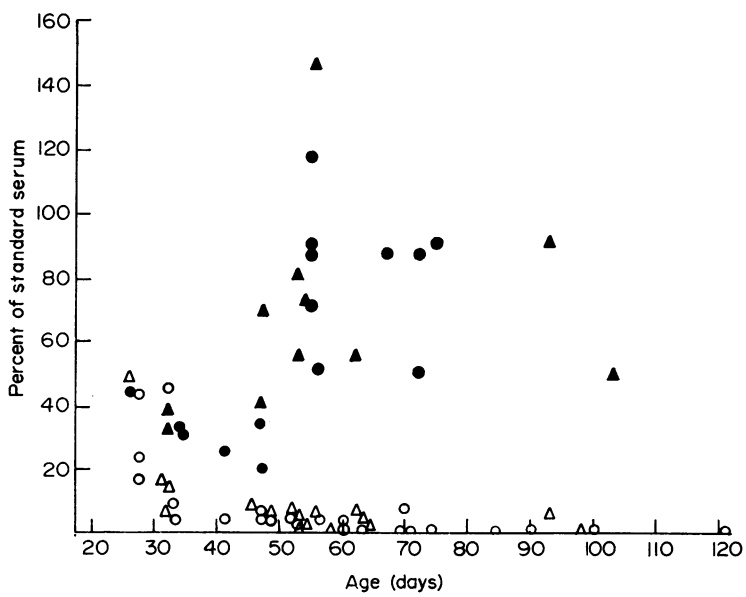

FIG. 3. Relationship of serum γG1 concentration to age in *nu/nu* and *nu/+* mice. ○, *nu/nu* male; △, *nu/nu* female; ●, *nu/+* male; ▲, *nu/+* female.

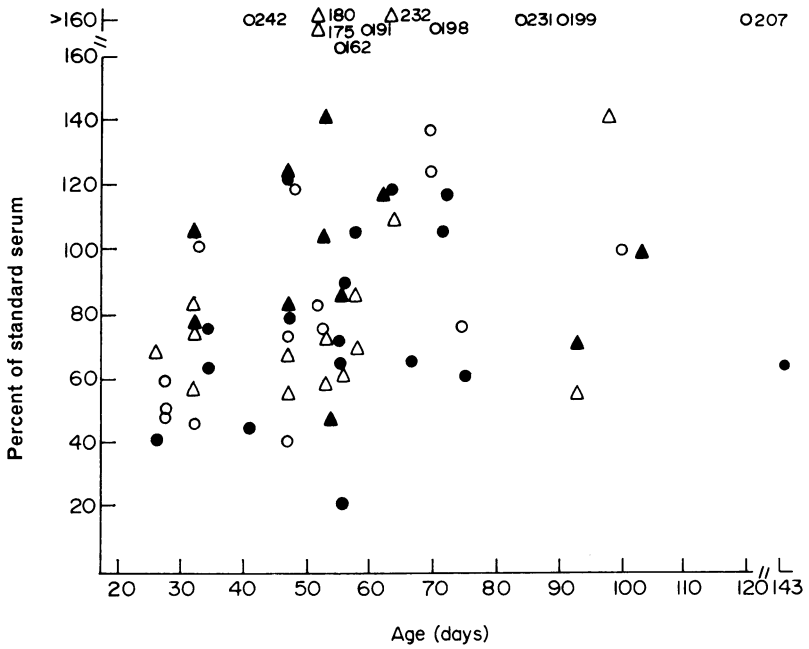

FIG. 4. Relationship of serum γM concentration to age in *nu/nu* and *nu/+* mice. ○, *nu/nu* male; △, *nu/nu* female; ●, *nu/+* male; ▲, *nu/+* female.

of life, it declined in *nu/nu* animals until in nine out of fourteen aged more than 60 days none could be detected. No differences were observed between male and female mice of either genotype.

Response to sheep erythrocytes

Although the concentration of γM in the serum of *nu/nu* mice was similar to, or even above, that in controls, the production of direct (presumed 19S) plaque-forming cells (PFC) in response to SRBC was impaired (Fig. 5). At the peak, 3 days after antigen administration, it was only 6% of the control response, although the subsequent decline in numbers was somewhat less rapid. Developed PFC, indicative of 7S antibody synthesis, were scarce or absent in *nu/nu* mice (Fig. 5). Concordant results were obtained from the haemagglutination assay (Fig 6). Mercaptoethanol-sensitive antibodies were detected in all immunized *nu/nu* mice, but at a low level. Only five of the *nu/nu* mice had detectable mercaptoethanol-resistant antibodies, which may be supposed to have belonged to one of the γG classes, whereas they were present in all *nu/+* animals assayed at day 5 or later. The addition of developing serum (Fig. 6b) increased the titres in *nu/+* mice by up to six $\log_2$ units. It had only a marginal effect on the titres of *nu/nu* sera, although on day 10 the titre of two sera was increased by four units. These data indicate an impairment of antibody formation, substantial for 19S antibody and profound for 7S, in response to primary exposure to SRBC. There does, nevertheless, appear to have been some synthesis of antibody molecules with 7S characteristics (mercaptoethanol-resistance and susceptibility to development); the present data do not identify to which class of immunoglobulin they belonged.

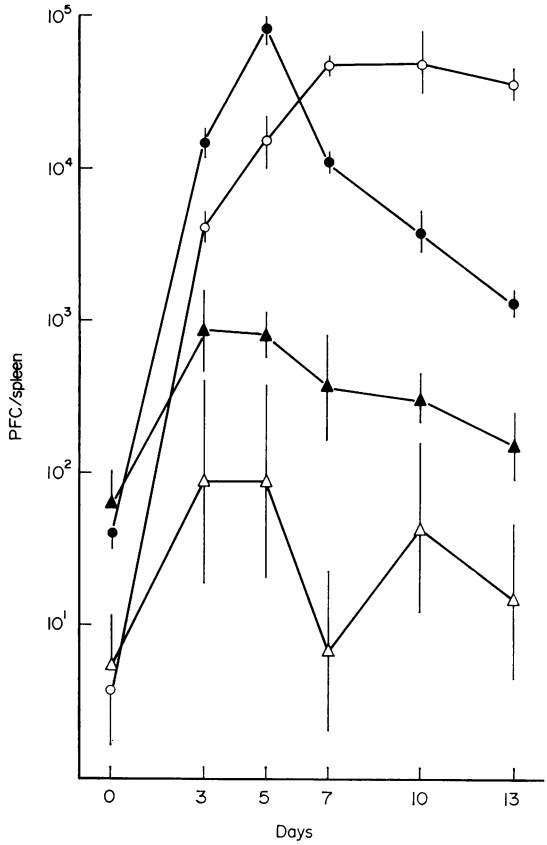

FIG. 5. Plaque-forming cells in the spleens of *nu/nu* and *nu/+* female mice at intervals after intraperitoneal injection of 4×10^7 SRBC. ▲, *nu/nu* direct PFC; △, *nu/nu* indirect PFC; ●, *nu/+* direct PFC; ○, *nu/+* indirect PFC. Three to four mice per point.

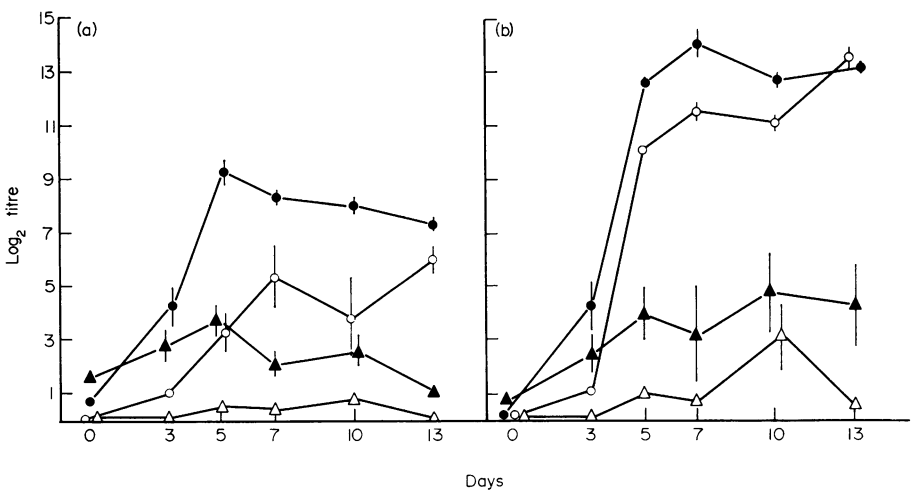

FIG. 6. Titres of haemagglutinating antibody in sera of *nu/nu* and *nu/+* female mice, after intraperitoneal injection of 4×10^7 SRBC. ▲, *nu/nu* total; △, *nu/nu* 2-ME resistant; ●, *nu/+* total; ○, *nu/+* 2-ME resistant. Three to four mice per point. (a) Before addition of developing antiglobulin serum. (b) After addition of developing antiglobulin serum.

Effects of thymus allografting

(a) *Survival.* A subcutaneous allograft of CBA/H neonatal thymus implanted at 10–21 days of age prolonged the survival of *nu/nu* mice, often considerably (Table 2). One animal is 500 days old at the time of writing. Nearly all animals killed and autopsied for experimental purposes up to 60 days after grafting had easily visible and cellular grafts. Some thymus-grafted mice appeared to gain weight more rapidly than controls, but weight-gain was in any case rather variable in *nu/nu* mice, and we have not examined the question in detail.

TABLE 2. Effect of subcutaneous thymus grafting* on survival of weaned *nu/nu* male and female mice†

	Percentage surviving at (weeks) :									
nu/nu mice (No.)	8	12	16	20	24	28	32	36	51	>70
Untreated (73)	78	29	11	7	3	0	0	0	0	0
Thymus-grafted (17)	100	65	47	35	29	24	18	12	6	6

* CBA neonatal thymus (one or two lobes) grafted at 10–21 days of age.
† Mice were weaned at 4 weeks of age; number of animals surviving at this time taken as 100%.

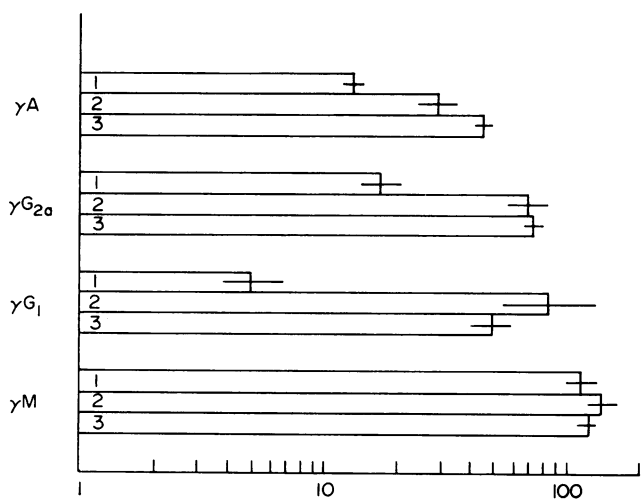

FIG. 7. Comparison of serum immunoglobulin levels in *nu/nu* and *nu/+* mice bled at 45–53 days of age. (Each bar represents the mean ± SE of six to eight sera; for statistical summary see Table 4). 1, *nu/nu* sham grafted; 2, *nu/nu* grafted subcutaneously at 10–18 days of age with one CBA neonatal thymus; 3, *nu/+* untreated.

(b) *Immunoglobulin concentrations.* The concentrations of immunoglobulins in *nu/nu* mice were tested at 35 and 42 days after thymus grafting and compared with *nu/+* and sham-operated *nu/nu* controls (Figs 7–8). A few mice were also tested after longer intervals (Table 3). All immunoglobulins except γM were found in amounts significantly ($P<0{\cdot}001$) above

TABLE 3. Serum immunoglobulin concentrations in long-surviving thymus-grafted* *nu/nu* and control individuals

Mouse	Genotype	Thymus graft	Age (days)	Percentage of standard serum γA	γG2a	γG1	γM
1a	*nu/nu*	−	91	17	11	<1	72
1b	*nu/nu*	+	91	52	79	57	40
2a	*nu/nu*	−	123	13	7	n.t.	91
2b	*nu/nu*	+	204	76	22	66	122
2c	*nu/+*	−	204	60	52	130	102
3a	*nu/nu*	−	63	14	27	2	127
3b	*nu/nu*	+	204	148	354	75	68
3c	*nu/+*	−	204	161	68	59	99
4a	*nu/nu*	−	81	28	39	2	338
4b	*nu/nu*	+	209	27	69	42	33
4c	*nu/+*	−	209	147	149	97	107

* CBA neonatal thymus (one lobe) grafted at 10–21 days of age.
n.t. = not tested.

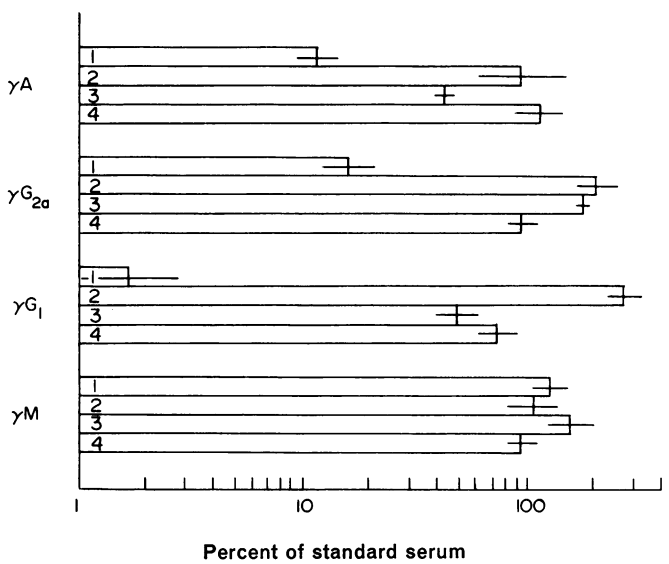

FIG. 8. Comparison of serum immunoglobulin levels in *nu/nu* and *nu/+* mice bled at 58–75 days of age. (Each bar represents the mean ± SE of four sera (three sera in group 3); for statistical comparison see Table 4). 1, *nu/nu* sham grafted or saline injected; 2, *nu/nu* injected intraperitoneally at 26–33 days of age with 10^8 CBA thymus cells; 3, *nu/nu* grafted subcutaneously at 16–25 days of age with one CBA neonatal thymus; 4, *nu/+* untreated.

those in *nu/nu* controls. γG2a and γG1 were raised in grafted animals to levels close to, or even above, those in *nu/+* controls, while γA tended to fall short of normal.

Intraperitoneally injected CBA/H thymus cells restored immunoglobulin concentrations even more effectively (Fig. 8). γA was up to the *nu/+* control level, while γG2a and γG1 were some two to three times higher than that. Treatment with a thymus graft or thymus cells did not significantly affect the concentration of γM in the serum.

These results are summarized in Table 4.

TABLE 4. Statistical comparison (Student's *t*-test) of immunoglobulin levels between *nu/nu* mice, grafted/injected with CBA thymus, and *nu/nu* and *nu/+* controls. (Further data in Fig. 7, Expt. 1 and Fig. 8, Expt. 2)

Expt. 1	γA	*nu/nu*	< <	*nu/nu*	(Tgr)*	<	*nu/+*
	γG2a	*nu/nu*	< < <	*nu/nu*	(Tgr)	=	*nu/+*
	γG1	*nu/nu*	< < <	*nu/nu*	(Tgr)	=	*nu/+*
	γM	*nu/nu*	=	*nu/nu*	(Tgr)	=	*nu/+*
Expt. 2	γA	*nu/nu*	< < <	*nu/nu*	(Tc)*	=	*nu/+*
		nu/nu	< <	*nu/nu*	(Tgr)	<	*nu/+*
	γG2a	*nu/nu*	< < <	*nu/nu*	(Tc)	>	*nu/+*
		nu/nu	< < <	*nu/nu*	(Tgr)	>	*nu/+*
	γG1	*nu/nu*	< < <	*nu/nu*	(Tc)	> >	*nu/+*
		nu/nu	< <	*nu/nu*	(Tgr)	=	*nu/+*
	γM	*nu/nu*	=	*nu/nu*	(Tc)	=	*nu/+*
		nu/nu	=	*nu/nu*	(Tgr)	=	*nu/+*

< < < : $P<0{\cdot}001$; < < : $P<0{\cdot}01$ < : $P<0{\cdot}05$; = : $P>0{\cdot}2$.
*Tgr: subcutaneous thymus graft. Tc: intraperitoneal thymus cells.

DISCUSSION

Quantitative analysis of sera from *nu/nu* mice 35–120 days old showed a marked depletion of γG1, γA and γG2a globulins. The amount of γM was normal or raised. This confirms and extends the finding of a low proportion of γ-globulin in the total plasma protein (Pantelouris, 1971), and is in general agreement with recent results of others (J.-C. Salomon & H. Bazin, personal communication; A. L. Luzzati & E. B. Jacobson, personal communication). Existing evidence suggests that *nu/nu* mice probably have normal B-lymphocyte populations (Wortis, 1971; Kindred, 1971b; Manning *et al.*, 1972). This, together with the fact that immunoglobulins were raised to near-normal levels after thymus grafting, suggests that the immunoglobulin deficiencies in untreated *nu/nu* mice are related to the thymic aplasia.

Such low serum levels of immunoglobulins could in principle result from any of the following causes: increased rate of catabolism, loss through the gastro-intestinal tract, and decreased rate of synthesis. Some neonatally thymectomized mice have been reported to show increased catabolism or loss of immunoglobulins; this appeared to be related to the presence of a wasting syndrome (Fahey *et al.*, 1965). It is possible that similar factors affect immunoglobulin levels in *nu/nu* mice. However, although these animals gain weight more slowly than normal littermates, they show little wasting except terminally and do not

usually have diarrhoea. A reduction in synthesis of the affected classes seems to be the most probable explanation of the results. Unstimulated axenic mice have been shown to have low serum concentrations of γG2 and γG1, which rise sharply after exposure to antigens (Benveniste, Lespinats, Adam & Salomon, 1971). This suggests that circulating γG-globulins are produced mainly in response to antigenic stimulation. Since the response to many antigens is known to be more or less dependent on the thymus, the synthesis of the corresponding antibodies would be depleted in *nu/nu* mice, and this depletion would be reflected in low circulating immunoglobulin concentrations.

The introduction of an allogeneic T-cell population, whether in the form of a graft or of a cell suspension, was followed by the appearance of much larger quantities of γA, γG2a and γG1 in the serum of *nu/nu* mice. Kindred (1971b) has suggested that some degree of genetic matching between thymus donor and host is necessary for long-term improvement of the antibody response to SRBC. In our experiments there was no deliberate matching, but it is possible that our *nu/nu* stock, which was originally from four pairs of animals, may by chance have a relatively high degree of histocompatibility with CBA. However, some selection in favour of histocompatibility with CBA may have occurred during the course of each experiment, since only a minority of thymus-grafted mice enjoyed an extensively prolonged lifespan.

Thymus grafts might exert their effect in any of three ways: by releasing donor-type T-cells, by providing a milieu for the maturation of host-type T-cells from bone marrow progenitors, or by exercising some hormonal influence on the peripheral lymphocyte population. Since thymus cells were at least as effective as thymus grafts in restoring immunoglobulin levels, the first mechanism may have been predominant under the conditions of the present experiments. The second may also have played a part, however, since *nu/nu* bone marrow undoubtedly contains progenitors capable of populating a thymus and producing functional T-cells (Wortis *et al.*, 1971; Pritchard & Micklem, 1973).

The reason why *nu/nu* mice injected with thymus cells tended to have supranormal levels of γG2a and γG1 is not clear. It seems unlikely to be related to any reaction by the injected cells against host antigens (such as was in principle possible), since Koltay *et al.* (1965) found graft-versus-host reactions to be associated with markedly reduced concentrations of circulating immunoglobulins. Furthermore, the clinical state of the animals gave no indication that a graft-versus-host reaction was in fact in progress.

The most striking deficiency of adult *nu/nu* mice was in γG1 globulin. The present experiments do not establish whether the relatively normal levels present at the time of weaning are derived from the mother or synthesized by the *nu/nu* mouse itself. The kinetics of disappearance with increasing age could fit the reported half-life for this protein of about 10 days (Bazin & Malet, 1969), and are compatible with the idea that no synthesis at all occurs after weaning. Nevertheless, the fact that introduction of a T-cell population is followed by large-scale synthesis indicates that B-cells capable of making γG1 under suitable conditions remain in *nu/nu* mice. The synthesis of γG1 thus appears to be peculiarly dependent on the presence of T-cells. This conclusion agrees with Torrigiani's (1972) data on the production of specific antibodies in mice thymectomized at 1 week of age. In axenic mice, on the other hand, Benveniste *et al.* (1969) found raised γG1 (and γG2) concentrations after neonatal thymectomy. The reason for this difference is not clear, but both the germ-free state of their animals and the probable presence of residual T-cells may contribute to it.

The ability of *nu/nu* mice to maintain normal or above-normal concentrations of serum γM is in marked contrast to their poor 19S antibody response to SRBC (also reported by Wortis (1971), Kindred (1971a) and Pantelouris & Flisch (1972)). Although it is possible that the γM does not represent responses to any exogenous stimuli, the bulk of it may well consist of specific antibodies to antigens such as bacterial capsular components, the response to which is relatively or completely thymus-independent. The presence of γM in axenic mice (Benveniste *et al.*, 1969) does not argue strongly against this supposition, since the diet of such mice, although sterile, is not antigen-free.

The profound depression of 7S antibody formation against SRBC provides direct confirmation of the inference which Pantelouris & Flisch (1972) drew from their data, and is concordant with the view (Taylor & Wortis, 1968) that T-cell deprivation particularly affects 7S antibody synthesis.

The results reported here demonstrate that T-cells, in addition to their well-known adjuvant function in the production of many specific antibodies, may also have a profound influence on the overall serum levels of γG and γA globulins. Although some effect on the catabolic rate of these proteins has not been excluded, it is considered that the most probable mode of T-cell influence is on synthesis.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council, of which Helen Pritchard is a Scholar. We are grateful to Mr A. C. Payne for his help with the haemolytic plaque assay and to Dr H. Bazin for verifying the specificity of the anti-γG1 serum.

REFERENCES

ARNASON, B.G., DE VAUX ST. CYR, C., SHAFFNER, J.B. (1964) A comparison of immunoglobulins and antibody production in the normal and thymectomized mouse. *J. Immunol.* **93,** 915.

BAZIN, H. & DUPLAN, J.F. (1966). Modifications du taux des immunoglobulines chez des souris thymectomisées à l'age adulte et irradiées. *Rev. Franç. Étude Clin. et Biol.* **11,** 987.

BAZIN, H. & MALET, F. (1969). The metabolism of different immunoglobulin classes in irradiated mice. I. Catabolism. *Immunology*, **17,** 345.

BAZIN, H., SAVIN, M.-C. & MICKLEM, H.S. (1968). Separation of mouse immunoglobulins on the basis of water-solubility on Sephadex G-25. *J. Chromatogr.* **34,** 180.

BENVENISTE, J., LESPINATS, G., ADAM, C. & SALOMON, J.-C. (1971) Immunoglobulins in intact, immunized, and contaminated axenic mice: study of serum IgA. *J. Immunol.* **107,** 1647.

BENVENISTE, J., LESPINATS, G. & SALOMON, J.-C. (1969). Study of immunoglobulins in axenic mice thymectomized at birth. *Proc. Soc. exp. Biol. (N.Y.)*, **130,** 936.

CLAFLIN, A.J., SMITHIES, O. & MEYER, R.K. (1966). Antibody responses in bursa-deficient chickens, *J. Immunol.* **97,** 693.

DRESSER, D.W. & WORTIS, H.H. (1967). Localized haemolysis in gel. In: *Handbook of Experimental Immunology* (Ed. by D. Weir), p. 1054. Blackwell Scientific Publications, Oxford and Edinburgh.

FAHEY, J.L., BARTH, W.F. & LAW, L.W. (1965) Normal immunoglobulin and antibody response in neonatally thymectomized mice. *J. nat. Cancer Inst.* **35,** 663.

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

HUMPHREY, J.H., PARROTT, D.M.V. & EAST, J. (1964) Studies on globulin and antibody production in mice thymectomized at birth. *Immunology*, **7,** 419.

JERNE, N.K., NORDIN, A.A. & HENRY, C. (1963) The agar plaque technique for recognizing antibody producing cells.: *Cell-Bound Antibodies* (Ed. by D. B. Amos and H. Koprowski), p. 109. Wistar Institute Press, Philadelphia.

KINDRED, B. (1971a) Immunological unresponsiveness of genetically thymus-less nude mice. *Eur. J. Immunol.* **1,** 59.

KINDRED, B. (1971b) Antibody response in genetically thymus-less nude mice injected with normal thymus cells. *J. Immunol.* **107,** 1291.

KOLTAY, M., KINSKY, R.G., ARNASON, B.G. & SHAFFNER, J.B. (1965) Immunoglobulins and antibody formation in mice during the graft-versus-host reaction. *Immunology*, **9,** 581.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2,** 235.

MANNING, J.K., REED, N.D., & JUTILA, J.W. (1972) Antibody response to *Escherichia coli* lipopolysaccharide and Type III pneumococcal polysaccharide by congenitally thymus-less (nude) mice. *J. Immunol.* **108,** 1470.

MISHELL, R.I. & DUTTON, R.W. (1967) Immunization of dissociated spleen cell cultures from normal mice. *J. exp. Med.* **126,** 423.

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.

PANTELOURIS, E.M. & FLISCH, P.A. (1972) Estimation of PFC and serum haemolysin response to SRBC in nude mice. *Immunology*, **22,** 159.

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

PORATH, J., AXEN, R. & ERNBACK, S. (1967) Chemical coupling of proteins to agarose. *Nature (Lond.)*, **215,** 1491.

PRITCHARD, H., & MICKLEM, H.S. (1972) Immune responses in congenitally thymus-less mice. I. Absence of response to oxazolone. *Clin. exp. Immunol.* **10,** 151.

PRITCHARD, H. & MICKLEM, H.S. (1973) Haematopoietic stem cells and progenitors of functional T-cells in the bone marrow of 'nude' mice. (In press).

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

REED, N.D. & JUTILA, J.W. (1972) Immune response of congenitally thymus less mice to heterologous erythrocytes. *Proc. Soc. exp. Biol. (N.Y.)*, **139,** 1234.

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

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.

TAYLOR, R.B. & WORTIS, H.H. (1968) Thymus dependence of antibody response: variation with dose of antigen and class of antibody. *Nature (Lond.)*, **220,** 927.

TORRIGIANI, G. (1972) Quantitative estimation of antibody in the immunoglobulin classes of the mouse. II. Thymic dependence of the different classes. *J. Immunol.* **108,** 161.

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

WORTIS, H.H., NEHLSON, S. & OWEN, J.J.T. (1971) Abnormal development of the thymus in 'nude' mice. *J. exp. Med.* **134,** 681.

WORTIS, H.H., TAYLOR, R.B. & DRESSER, D. W. (1966) Antibody production studied by means of the L.H.G assay. I. The splenic response of CBA mice to sheep erythrocytes. *Immunology*, **11,** 603.