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Frequencies of background cytoplasmic Ig-containing cells in various lymphoid organs of athymic and euthymic mice as a function of age and immune status

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Summary. The distribution of background Ig-secreting cells, measured as cells containing cytoplasmic immunoglobulin (C-Ig cells), over spleen, bone marrow, lymph nodes and Peyer's patches was studied in congenitally athymic (nude) mice and heterozygous euthymic mice as a function of age and immune status (germ-free (GF) *vs* specific pathogen-free (SPF)). In young athymic as well as in young euthymic mice, the spleen was found to contain the great part of all C-Ig cells, irrespective of whether the mice were GF or SPF. The number of C-Ig cells in the spleen was found to be rather constant over the life span, while the number of C-Ig cells in the bone marrow of all groups of mice greatly increased with age. This indicates that the relative shift of C-Ig cells to the bone marrow is neither dependent on the presence of the thymus, nor on the microbiological status of the mice. However, at young and intermediate age the microbiological status of the mice did affect the total number of C-Ig cells per mouse. This was mainly due to the effect upon the bone marrow, mesenteric lymph nodes and Peyer's patches. At these ages the background Ig synthesis in these organs appeared to be mainly dependent on external antigenic stimulation, in contrast to the spleen, where the Ig synthesis appeared to be mainly due to endogenous stimulation. The Ig (sub)class distribution of the C-Ig cells was different for all different organs tested. Hardly or no difference in percentage distribution was found between the GF nude and GF heterozygous mice. Most C-Ig cells in spleen, bone marrow and lymph nodes of the GF mice were of the IgM isotype. C-IgG and C-IgA cells occurred in substantial percentages only in bone marrow and lymph nodes. In the lymph nodes of GF nude mice a remarkably high percentage of C-IgA cells was found.

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INTRODUCTION

Serum and secretory immunoglobulin (Ig) levels vary with genetic background, antigenic load and age (Wostmann, 1975; Van Snick & Masson, 1980; Benner & Haaijman, 1980; Radl, 1981). These levels are built up and maintained by the Ig-secreting cells in the various lymphoid organs and, in some instances, at secretory sites. To get insight into the regulation of the 'spontaneous' (background) Ig production it is important to know the distribution of the Ig-secreting cells over the various lymphoid organs, the origin of these cells, their Ig (sub)class distribution and specificity repertoire, and the stimuli which initiate their generation.

By using the technique of cytoplasmic immunofluorescence on cytocentrifuge preparations, the organ distribution and the Ig class distribution have been studied in man (Hijmans, 1975; Turesson, 1976), chicken (Lawrence *et al.*, 1979) and mouse (reviewed in Benner *et al.*, 1982).

The main data emerging from our previous studies of mice are that (a) after half a year of age the majority of all C-Ig cells of a mouse resides in the bone marrow (Haaijman, Schuit & Hijmans, 1977); (b) in nude mice C-IgG and C-IgA cells are highly deficient in spleen and bone marrow, but not in mesenteric lymph nodes and Peyer's patches (Haaijman *et al.*, 1979); (c) the deficiency of C-IgG and C-IgA cells in nude mice is compensated for by increased numbers of C-IgM cells (Haaijman *et al.*, 1979); (d) the age-related increase of the total C-Ig cell number in the bone marrow of nude mice is retarded as compared with thymus-bearing littermates (Haaijman *et al.*, 1979; (e) germ-free (GF) mice have lower C-Ig cell numbers than specific pathogen-free (SPF)-reared mice, especially in the bone marrow, mesenteric lymph nodes and Peyer's patches (Haaijman *et al.*, 1977); and (f) frequencies of C-Ig cells are highly dependent on both the degree of external antigenic stimulation and the presence of T cells (Benner *et al.*, 1981a).

In the present study we have proceeded along this way. Now we report data on the combined influence of GF maintenance and congenital absence of the thymus upon the number and organ distribution of C-Ig cells at various ages. Therefore, we determined frequencies of C-IgM, C-IgG_1, C-IgG_2, C-IgG_3 and C-IgA cells in various lymphoid organs of GF athymic nude mice. The data are compared with similar frequency determinations in SPF athymic nude mice and GF and SPF euthymic mice.

MATERIALS AND METHODS

Mice

Female germ-free (GF) and specific pathogen-free (SPF) nude mice on a BALB/c background and their heterozygous littermates, 6–8, 40 and 100-week-old were purchased from the Radiobiological Institute TNO, Rijswijk (zh), The Netherlands. All mice were barrier-maintained and received sterilized pelleted food (Hope Farms, Woerden, The Netherlands) and sterilized water *ad libitum*. All mice tested were free of lymphoreticular malignancies.

Cytocentrifuge preparations

Cell suspensions of spleen, femoral bone marrow, mesenteric lymph nodes and Peyer's patches were prepared as described in detail in a previous paper (Haaijman *et al.*, 1979). Cytocentrifugre preparations of a known number of cells were prepared according to Vossen *et al.* (1976).

Visualization of cytoplasmic Ig-containing cells

The technique for the visualization of cytoplasmic Ig-containing cells (C-Ig cells) has been described in detail by Hijmans, Schuit & Klein (1969). Slides were fixed for 15 min at $-20°$ in acid ethanol (5 parts of acetic acid to 95 parts of ethanol) and washed for 30 min in PBS. Incubations with fluorescent antisera were performed for 30 min in a humid chamber, Excess conjugate was removed with one wash of PBS. Preparations were mounted in buffered glycerol (1 part of PBS to 9 parts of glycerol, p.a., Merck-Schuchard, München, FRG) and sealed with paraffin. The Ig class distribution of the C-Ig cells was determined by using combinations of rhodamine- and fluorescein-labelled antisera specific for the heavy chains of IgM, IgG_1, IgG_2, IgG_3 and IgA. The fluorescence microscope equipment has also been described in a previous paper (Haaijman *et al.*, 1979). Cytoplasmic Ig-positive cells could easily be discriminated from pre-B cells. The latter only have a small rim of cytoplasmic Ig and show a faint staining in immunofluorescence (Raff *et al.*, 1976).

Conjugated antisera

A fluorescein-conjugated goat antiserum directed against mouse Igs (GAM-Ig/FITC; lot No. 2-873, Nordic Immunological Laboratories, Tilburg, The Netherlands) was used to determine the total number of C-Ig cells per slide.

The Ig class distribution of the C-Ig cells was determined according to Hijmans *et al.* (1969) using combinations of rhodamine- and fluorescein-labelled antisera specific for the heavy chains of IgM, IgG_1, IgG_2, IgG_3 and IgA. The rhodamine- and fluorescein-labelled antisera, specific for the heavy chains of murine IgM, IgG_1, IgG_2 and IgA were prepared, purified and generously supplied by Dr J. Radl and Miss P. van den Berg from the Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands. The antiserum specific for the heavy chain of

murine IgG_3 was purchased from Nordic Immunological Laboratories (lot No. 11-478). The antisera fulfilled all specificity criteria described previously (Haaijman *et al.*, 1979). The specificity of the reagents was corroborated by the fact that preparations of quite a number of normal young adult BALB/c mice did not show any C-Ig cell containing Ig of two different (sub)classes, notably in mesenteric lymph nodes and Peyer's patches.

Calculation of the number of C-Ig cells per organ

The total number of C-Ig cells per organ was calculated from the number of cells spun down per slide and the cell yield per organ. We have used a conversion factor of 7·9 to calculate the number of C-Ig cells in the total bone marrow from the C-Ig cell number found in two femurs (Benner, van Oudenaren & Koch, 1981b). For calculation of the number of C-Ig cells in the total lymph node tissue we have determined C-Ig cell numbers in the mesenteric lymph nodes, and estimated the total lymph node mass of a mouse at the 3-fold of the mesenteric lymph nodes. The percentage of C-Ig cells did not differ significantly between peripheral and mesenteric lymph nodes. For determination of the number of C-Ig cells in the Peyer's patches all patches from the small intestine were collected, avoiding inclusion of the gut wall.

RESULTS

C-Ig cells in different lymphoid organs

The total number of C-Ig cells in spleen, bone marrow, lymph nodes and Peyer's patches of GF and SPF athymic nude (nu/nu) mice and their heterozygous littermates (nu/+) mice are depicted in Fig. 1. From both groups of mice, GF- and SPF-raised animals were tested at three different ages namely, at 6–8, 40 and 100 weeks of age.

The absolute number of C-Ig cells in the spleen of GF and SPF nu/nu and nu/+ mice was found to be rather constant over the lifespan tested. Only the 100-week-old GF and SPF nu/+ mice displayed considerably more C-Ig cells in the spleen than all other groups of mice. The percentage contribution of the spleen to the total number of C-Ig cells per mouse varied greatly over the lifespan. In 6–8-week-old mice, this percentage varied between 50 and 80%. In the 40-week old GF and SPF heterozygous mice, the contribution of the spleen had already decreased till about 16%. In nude mice of the same age, the contribution of the spleen was approximately 35%. In the various groups of 100-week-old mice, the contribution of the spleen varied between 16 and 26% (Table 1).

At young age, the bone marrow of the GF nu/nu and nu/+ mice contributed about 34 and 18% to the total number of C-Ig cells per mouse, respectively. In

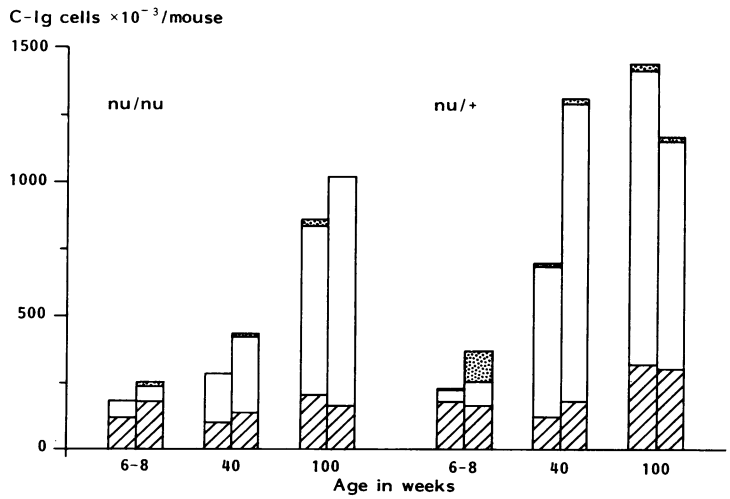

Figure 1. Number of C-Ig cells in mouse spleen (*hatched area*), total bone marrow (*open area*) and total lymph node tissue and Peyer's patches together (*dotted area*) of 6–8-, 40- and 100-week-old GF and SPF nu/nu and nu/+ mice. From each pair of columns the left one represents the GF mice and the right one the SPF mice. Whenever the lymph nodes and Peyer's patches contributed less than 3000 C-Ig cells, this contribution is not drawn in the figure.

SPF mice this contribution was just between these percentages. With age the percentage contribution of the bone marrow became larger. Initially this was the most prominent in the GF and SPF heterozygous mice, but at the age of 100 weeks the contribution of the bone marrow of the GF and SPF nude mice had also considerably increased. In the various groups of 100-week-old mice the percentage contribution of the bone marrow varied from 74% in GF nude mice to 76% in GF nu/+ mice.

In the lymph nodes and Peyer's patches an appreciable number of C-Ig cells was only found in the youngest age group of SPF nu/+ mice and constituted about 23% of the total number of C-Ig cells. In all other groups studied we never found a higher contribution for the total lymph node tissue than 1 or 2% of the total number of C-Ig cells (Table 1).

The Ig class distribution of the C-Ig cells in spleen, bone marrow and lymph nodes of GF nu/nu and nu/+ mice are presented in Tables 2, 3 and 4,

Table 1. Number of C-Ig cells in spleen, bone marrow, lymph nodes and Peyer's patches together of GF and SPF nu/nu and nu/+ mice

Mice	Status	*n*	age (weeks)	Spleen	Bone marrow	Lymph nodes	Peyer's patches	Total C-Ig cells per mouse*
nu/nu	GF	4	6–8	123±24(65)†	64±18(34)	1·1±0·3(<1)	0·2±0·04(<1)	188±43
nu/nu	SPF	6	6–8	185±36(76)	53±6(22)	4·5±1·1(2)	0·8±0·2(<1)	244±44
nu/+	GF	5	6–8	182±16(81)	41±5(18)	1·6±0·4(<1)	1·2±0·3(<1)	226±22
nu/+	SPF	10	6–8	169±35(52)	83±15(25)	24±10(7)	51±15(16)	327±75
nu/nu	GF	9	40	106±16(37)	178±20(62)	1·0±0·3(<1)	0·4±0·1(<1)	285±37
nu/nu	SPF	8	40	140±16(33)	288±27(67)	1·5±0·4(<1)	0·3±0·1(<1)	430±46
nu/+	GF	4	40	126±51(18)	560±110(81)	3·0±1·0(<1)	0·7±0·2(<1)	690±164
nu/+	SPF	6	40	181±33(14)	1108±119(86)	5·0±2·3(<1)	0·8±0·2(<1)	1295±162
nu/nu	GF	6	100	207±35(25)	628±92(74)	7·5±4·0(<1)	—‡	844±132
nu/nu	SPF	5	100	166±35(16)	854±249(84)	1·1±0·4(<1)	—	1021±288
nu/+	GF	6	100	325±75(23)	1086±193(76)	8·6±2·1(<1)	—	1421±268
nu/+	SPF	13	100	306±42(26)	842±125(73)	—	14±6(1)	1162±172

* Total of C-Ig cells positive for IgM, IgG and IgA in all lymphoid organs together.
† Arithmetic mean±SEM. In brackets the percent contribution of the total number of C-Ig cells per mouse.
‡ The indicated organ was not macroscopically visible in this particular group of mice.

Table 2. Ig-class distribution of C-Ig cells in the spleen of young and old GF nu/nu and nu/+ mice

		C-Ig cells (%)							
Mice	Age (weeks)	IgM	IgG_1	IgG_2	IgG_3	IgA	M+G_1*	M+G_2	M+G_3
nu/nu	6–8	87±3†	2±1	0·4±0·2	0·4±0·3	1±0·2	2±1	3±1	3±0·4
nu/+	6–8	85±2	0	1±0·3	0·3±0·2	1±0·1	2±1	7±1	3±1
nu/nu	100	87±2	3±1	1±0·2	0·1±0·1	2±1	2±1	4±1	1±0·2
nu/+	100	85±3	0·2±0·2	1±0·4	1±0·5	1±0·2	2±1	9±4	1±0·5
		C-Ig cells $\times 10^{-3}$/organ							
		IgM	IgG_1	IgG_2	IgG_3	IgA	M+G_1	M+G_2	M+G_3	Total
nu/nu	6–8	107±22	3±1	0·5±0·3	1±0·4	2±0·4	2±1	4±0·5	4±1	123±24
nu/+	6–8	156±15	0	1±0·5	1±0·4	2±0·2	4±1	13±2	6±1	182±16
nu/nu	100	178±28	7±3	2±1	0·3±0·3	4±2	4±1	11±4	2±0·3	207±35
nu/+	100	281±69	1±1	3±1	4±2	3±1	5±2	24±7	4±1	325±75

* In the combinations M stands for IgM, G for IgG and A for IgA.
† Average percentage of Ig class±SEM. The same GF mice were tested as used for Table 1 and Fig. 1.

Table 3. Ig-class distribution of the C-Ig cells in the bone marrow of young and old GF nu/nu and nu/+ mice*

		C-Ig cells (%)							
Mice	Age (weeks)	IgM	IgG_1	IgG_2	IgG_3	IgA	M+G_1	M+G_2	M+G_3
nu/nu	6–8	73±6	6±4	2±1	3±2	11±3	1±1	3±1	2±1
nu/+	6–8	67±4	0	3±1	6±2	6±1	2±2	10±3	6±3
nu/nu	100	71±7	11±4	8±4	2±0·3	4±1	1±0·4	3±1	1±0·4
nu/+	100	73±3	2±0·5	2±1	2±1	7±1	1±1	12±4	1±0·5
		C-Ig cells $\times 10^{-3}$/organ							
		IgM	IgG_1	IgG_2	IgG_3	IgA	M+G_1	M+G_2	M+G_3	Total
nu/nu	6–8	46±10	6±5	1±1	1±1	7±3	0·3±0·2	2±1	1±0·5	64±18
nu/+	6–8	27±4	0	1±0·4	2±1	3±1	1±1	4±1	3±1	41±5
nu/nu	100	451±76	69±31	47±28	12±4	21±6	3±3	20±10	5±2	628±92
nu/+	100	779±127	19±7	19±7	32±14	72±13	7±4	150±62	8±8	1086±193

* Details and abbreviations as in Table 2.

Table 4. Ig-class distribution of the C-Ig cells in the total lymph node tissue of young and old GF nu/nu and nu/+ mice

		C-Ig cells (%)							
Mice	Age (weeks)	IgM	IgG_1	IgG_2	IgG_3	IgA	M+G_1	M+G_2	M+G_3
nu/nu	6–8	50±12	0·5±0·5	10±2	8±5	27±7	0	1±1	4±1
nu/+	6–8	64±4	4±1	11±7	4±2	9±3	1±1	8±4	0
nu/nu	100	46±4	6±4	11±2	5±2	26±5	0	5±1	1±1
nu/+	100	57±5	0	8±1	14±2	11±3	0	8±2	2±1
		C-Ig cells $\times 10^{-3}$/organ							
		IgM	IgG_1	IgG_2	IgG_3	IgA	M+G_1	M+G_2	M+G_3	Total
nu/nu	6–8	1·5±0·6*	<0·1	0·3±0·1	0·3±0·1	1±0·5	0	<0·1	0·3±0·1	3±1
nu/+	6–8	3±0·6	0·3±0·1	1±0·3	0·3±0·1	1±0·3	<0·1	1±0·3	0	6±1
nu/nu	100	12±6	1±1	3±2	1±1	5±1	0	1±0·3	0·3±0·1	23±12
nu/+	100	15±3	0	2±0·3	4±1	3±2	0	2±1	1±0·3	27±6

* The number of C-Ig cells per total lymph node tissue of a mouse was estimated at 3 times the number found in the mesenteric lymph nodes. See Table 2 for other details and for abbreviations.

respectively. These latter tables also give percentages and numbers of cells containing two different Ig heavy chains. At 6–8 as well as at 100 weeks of age, 85% or more of the C-Ig cells were of the IgM class (Table 2). Of the other C-Ig cells in the spleen 2–4% contained IgG only, and 1–2% IgA only. The other C-Ig cells were 'double producers', mostly containing the IgM and IgG_2 isotypes. These double-positive cells were more frequent in nu/+ mice than in the nu/nu mice.

In bone marrow of 6–8 and 100-week-old GF nu/nu and nu/+ mice, about 70% of the C-Ig cells contained IgM only (Table 3). C-Ig cells, containing IgG only were more frequent in nude mice than in nu/+ mice, whereas cells positive for IgM as well as for one of the subclasses of IgG were more frequent in nu/+ mice. In absolute numbers C-Ig cells of all (sub)classes increased considerably in the bone marrow from 6–8 to 100 weeks of age. In nu/nu mice this was the most prominent for C-IgM and C-IgG_2 cells, and in nu/+ mice for C-IgM and C-IgA cells. In the bone marrow

of 100-week-old GF nu/+ mice 150,000 double-positive C-IgM/IgG$_2$ cells were found.

In the lymph nodes of young and old GF nude mice about 50% of the C-Ig cells were found to contain IgM. For their heterozygous littermates this figure was about 60% (Table 4). C-IgG$_2$ and C-IgG$_3$ cells were the most prominent C-IgG cells in the lymph nodes of young and old GF nu/nu and nu/+ mice. Remarkably, C-IgA cells were 2–3 times more frequent in the young and old nude mice than in the heterozygous littermates. C-Ig cells containing two different heavy chain isotypes were most frequently C-IgM/IgG$_2$-positive cells.

In GF nu/nu and nu/+ mice, macroscopically visible Peyer's patches were found in young animals only. However, the number of C-Ig cells was always very small, i.e. < 1000/mouse. In the 6–8-week-old nude mice we found among these C-Ig cells 27% C-IgM cells, 2% C-IgG$_2$ cells and 10% C-IgG$_3$ cells. The most prominent isotype of the C-Ig cells in the Peyer's patches of GF nude mice was IgA. These C-IgA cells constituted about 60% of all C-Ig cells. In the GF heterozygous littermates we found 35% C-IgM cells, 18% C-IgG$_2$ cells and 30% C-IgA cells.

Comparing the class distribution of the C-Ig cells in the various lymphoid organs of GF nu/nu and nu/+ mice (Tables 2–4) on one hand, and of SPF nu/nu and nu/+ mice (Haaijman *et al.*, 1979) on the other, we found that in the spleen of young and old GF nu/nu and nu/+ and young and old SPF nu/nu mice the percentage of C-IgG cells was rather the same—between 2 and 6%— whereas in 6- and 100-week-old SPF nu/+ mice the percentage of C-IgG cells was 32 and 22%, respectively. The same phenomenon was observed for the IgA class and for the other organs tested. This implies an increased switch frequency from IgM to IgG and IgA in SPF nu/+ mice.

DISCUSSION

The aim of this report is to define the factors which determine the number and organ distribution of 'spontaneously' occurring (background) Ig-containing cells in the lymphoid system of ageing mice. The results presented show that the immune status (GF *vs* SPF and nu/nu *vs* nu/+) is an important factor determining the total number of C-Ig cells as well as the distribution of these C-Ig cells over the various lymphoid organs. The more the immune system is stimulated by external antigens, the higher the total number of C-Ig cells/mouse. This, however, was completely due to the occurrence of higher numbers of C-Ig cells in bone marrow, lymph nodes and Peyer's patches, as the number in the spleen was quite constant in all groups of mice.

In previous studies we have shown that the complete deprivation of mice from external antigenic stimulation by feeding GF mice a synthetic diet composed of low molecular weight compounds even completely prevented the development of macroscopically visible Peyer's patches and the appearance of C-Ig cells in the mesenteric lymph nodes (Benner *et al.*, 1981a). In absolute numbers, the decrease of C-Ig cells after antigen deprivation was most prominent in the bone marrow, which is the major site of C-Ig cell localization during adult life (Haaijman *et al.*, 1977; Haaijman *et al.*, 1979; Benner *et al.*, 1982). At young age, however, the spleen is the major site of localization of C-Ig cells. The age-related shift of the major proportion of C-Ig cells from the spleen to the bone marrow appeared to be neither dependent on the presence of the thymus, nor on the microbiological status of the mice. However, in GF athymic mice the accumulation of C-Ig cells in the bone marrow was retarded as compared to SPF heterozygous euthymic mice.

The occurrence of Ig-secreting cells within the bone marrow deserves special discussion. The marrow by itself is unable to mount an antibody response upon external antigenic stimulation at least not in response to a T-dependent antigen (Benner & van Oudenaren, 1975). We have recently shown that the Ig-secreting cells in the marrow are derived from the peripheral lymphoid organs (Koch *et al.*, 1981). Antigen-activated B cells can leave spleen and lymph nodes during the early phase of the response and migrate via the bloodstream towards the bone marrow, where they further mature into antibody-secreting plasma cells (Benner, van Oudenaren & de Ruiter, 1977). However, this occurs only during secondary type responses because only B memory cells and/or their progeny have this capacity (Koch *et al.*, 1981). This mechanism of antibody formation in the bone marrow can explain the age-related increase of Ig-secreting cells in this organ. The more an individual ages, the more often a certain antigenic encounter will have been experienced previously, leading to a secondary type response which involves the bone marrow.

It is of interest that the number of splenic C-Ig cells hardly varied in these studies (SPF *vs* GF; nu/nu *vs* nu/+). Taken together, our studies suggest that the

background Ig synthesis in the bone marrow, mesenteric lymph nodes and Peyer's patches is mainly dependent on external antigenic stimulation, whereas the background Ig synthesis in the spleen is mainly due to endogenous stimulation. These stimuli might be derived from idiotypes (Cosenza, 1976), from autologous erythrocytes (Steele & Cunningham, 1978) and from certain serum components (Grabar, 1979). Thus, prolonged low-level endogenous stimuation might, in the long run, induce the generation of B memory cells and reactivate these cells, and thereby induce Ig synthesis in the marrow.

Immunization of nude mice can lead to the generation of B memory cells and the occurrence of antibody-forming cells in the bone marrow (Benner, van Oudenaren & Haaijman, 1978). However, this bone marrow antibody-forming cell response in nude mice is lower than in heterozygous mice. These data, together with the present observations that the presence of the thymus is not obligatory to the age-related shift of the majority of the C-Ig cells from spleen to bone marrow, and that the age-related accumulation of C-Ig cells in the marrow of nude mice is retarded, suggest that nude mice mature immunologically more slowly than heterozygous mice. This can also explain why the C-Ig cell numbers in the bone marrow of very old nude mice are quite comparable to those of very old heterozygous mice (Fig. 1).

A significant finding in this report is the observation that, while nude mice have less immunocytes containing IgG and IgA, they have more immunocytes containing IgM. This is in accordance with the report of Bankhurst, Lambert and Miescher (1975) containing serum Ig levels in nude mice. While the levels of IgG and IgA were decreased, that of IgM was increased as compared to nu/+ mice. Apparently, under conditions of decreased helper T cell activity, the immune system can compensate for the lack of thymus-dependent (IgG and IgA) antibody formation with increased thymus-independent IgM antibody production.

The Ig class distribution of the C-Ig cells, especially the occurrence of C-IgG and C-IgA cells in spleen, bone marrow and lymph nodes of GF mice, appeared to be highly dependent on the age of the mice studied and the availability of T cells (Tables 2–4). Since the expression of different surface Ig isotypes does not vary between thymus-bearing conventional mice, athymic nude mice and GF mice (Abney *et al.*, 1978; Lawton & Cooper, 1974), these decreased numbers of C-IgG and C-IgA cells must be due to a limited production of IgG- and IgA-secreting cells from mature B precursor cells. Remarkably, the number of C-IgG and C-IgA cells in the spleen of GF nude mice was as low as in GF nu/+ mice, whereas we did find a small but distinct difference in the bone marrow and lymph nodes of both groups of mice. In the bone marrow of old GF nude mice we found about 2–3 times the C-IgG_1 and C-IgG_2 cells than in the marrow of old GF nu/+ mice. The high number of C-IgG_1 cells in nude mice is in contrast with studies about the high T-dependence of the IgG_1 synthesis (Taylor & Wortis, 1968; Martinez-Alonso, Coutinho & Augustin, 1980; Coutinho *et al.*, 1982). The number of C-IgG_3 cells in the bone marrow of old GF nu/+ mice was about 3 times higher than in old GF nude mice, so that the most frequent IgG isotype in old nu/+ mice was IgG_3.

In the mesenteric lymph nodes of GF mice, a deficiency of C-IgA cells was not apparent. This suggests that in the intestine the maturation of IgA-committed B cells to C-IgA cells is mainly stimulated by the antigenic stimuli of the food. We found in young and old nude mice a 2–3 times higher percentage of C-IgA cells than in the heterozygous littermates. This is remarkable in view of the reported T dependence of IgA synthesis (Taylor & Wortis, 1968; van Muiswinkel & van Soest, 1975). On the other hand, increased serum IgA levels have been reported for neonatally thymectomized mice (Fahey, 1965). Therefore the actual IgA-synthesis is determined by both the extent of T cell deprivation and the antigenic load. Under particular conditions of decreased T cell help, a certain antigenic load might evoke a stronger antigenic stimulation of the B cells that do respond, leading to a higher switch frequency to IgA.

The occurrence of a substantial percentage of C-Ig cells containing two different Ig heavy chain isotypes, especially IgM and IgG_2, was striking. In a previous paper we have extensively discussed this phenomenon (Benner *et al.*, 1981a) which is most likely related to the proliferative activity in the Ig-synthesizing cell compartment.

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