

## Characterization of Rat Bone Marrow Cells

### II. Analysis of Surface Antigens in Small Lymphocytes with Particular Reference to Thymus Antigen-Carrying Cells

K. ZEILLER AND E. HANSEN

*Max-Planck-Institut für Biochemie, Am Klopferspitz, 8033 Martinsried bei München, West Germany*

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Rat bone marrow (BM) small cells were enriched by velocity sedimentation, further separated by means of free-flow electrophoresis, and characterized using T- and B-cell-specific surface markers. More than 80% of these cells were small lymphocytes by morphological criteria and reacted with lymphocyte-specific antisera. A minority of cells had high electrophoretic mobility (EPM), carried surface antigens characteristic of mature T cells, and lacked B-cell markers. These cells may represent recirculating T cells. A small number of cells were found with rat B lymphocyte-specific antigen (RBLA) and surface immunoglobulin (sIg) and had medium EPM. These cell fractions also contained "null" cells which were devoid of T- and B-cell-specific antigens. More than 80% of the BM small cells had low EPM and carried the three subspecificities of the Thy-1 antigen complex and the Thy-A antigens. These antigens were found at several-fold higher concentration on the surface of all thymocytes, but are lacking in most other lymphocytes. The thymus antigen-carrying BM cells of low EPM do not carry other T- and B-cell-specific markers found in thymocytes and peripheral T and B lymphocytes. These markers comprise the T-cell antigens RTLA (rat T-lymphocyte-specific antigen) and RHLA (antigens specific for rat T cells of high EPM) and the B-cell markers RBLA and sIg. Thus the majority of rat BM lymphocytes differ from all other lymphocytes of the T- and B-cell series which makes any classification on this basis purely speculative.

### INTRODUCTION

The morphologically characterized lymphoid cells in the mammalian bone marrow comprise a mixture of stem cells, precursor cells and newly generated and recirculating mature lymphocytes. It would be of great advantage if these cells could be classified by better defined markers such as surface antigens, receptors, and cytophysical properties. In the mouse it has been shown that only a small number of lymphoid cells are recirculating T and B cells whilst most of the lymphocytes are "null" cells and surface Ig-positive B lymphocytes, which are continuously provided by marrow precursor cells. The majority of the "null" cells rapidly acquire B-cell surface markers and emigrate in a random fashion to the periphery (1-5). In the rat the situation is somewhat different since more than 80% of the BM lymphoid cells carry thymus-specific surface antigens and only a minority are "null" cells or show B-cell markers (6-8). The function as well as the T- and B-cell relationship of these cells is not yet clear. Hunt *et al.* (9) observed that

rat B cells develop from thymus antigen-carrying sIg<sup>-</sup> BM cells. Others, however, have argued that these cells belong to the T-cell lineage perhaps representing prethymocytes (6, 7). In this work it will be further shown that the thymus antigen-carrying rat BM lymphocytes which are small sized (10) represent a cell population which, on the basis of cytophysical properties and various surface markers, is different from all other lymphocytes so far classified into either the T- or B-cell lineage.

## METHODS

*Animals and cells.* Three-month-old female SPF inbred W/Neu rats (Institut für Strahlenforschung und Umweltschutz, Neuherberg, Munich) were used. Spleen, lymph node, and thymus (adherent lymph nodes had been carefully removed) were gently teased apart with needles and bone marrow tissue was dispersed by vigorous aspiration with a pipet. The cells were collected in TC solution Puck G (Difco, Detroit) supplemented with 1% w/v bovine serum albumin (BSA, Armour, Chicago) which had been previously deionized with Amberlite MB-3 (Serva, Heidelberg). After removal of residual tissue by filtration through thin layers of cotton wool the cells were washed twice with Puck BSA, using centrifugation at 80g for 10 min, and the final cell pellets resuspended in the medium used in the following experiment. All experiments were performed at 2–4°C.

*Corticosteroid treatment.* Rats were injected intraperitoneally with 6 mg 1,1-dihydroprednisone (Lentia, München) (prednisolone) per 100 g body weight. Lymphoid tissues were harvested 48 hr later.

*Velocity sedimentation.* Cells were separated by means of velocity sedimentation according to Miller and Phillips (11) using a slightly modified technique as described (12). The sedimentation profiles were corrected for viable nucleated cells evaluated using the trypan blue exclusion test. The resulting sedimentation profiles were standardized to a relative area as 100%.

*Free-flow electrophoresis.* The cells were separated with a free-flow electrophoretic apparatus FF IV (Bender und Hobein, München; DESAGA, Heidelberg) under conditions described in details elsewhere (13). The cell concentration per fraction was determined and corrected for viable cells evaluated using trypan blue exclusion test. The electrophoretic profiles were standardized to a relative area as 100%.

*Antilymphocyte sera.* Rabbit anti-rat lymphocyte subclass-specific antisera and anti-Ig antisera were prepared as described in detail recently (14). The different specificities (IgG fractions) were directed against the following differentiation antigens: RBLA (rat B-lymphocyte-specific antigens); R-Thy-1 antigens (Thy-1 antigens shared by all rat and mouse strains); R-Thy-1.1 antigens (Thy-1 antigens shared by all rat strains and mouse Thy-1.1 strains); R<sub>s</sub>-Thy-1 antigens (rat specific Thy-1 antigens which are lacking in all mice); R-ThyA (rat thymocyte-specific antigens different from R-Thy-1); RTLA (rat T-lymphocyte-specific antigens present on all thymocytes and peripheral T lymphocytes); RHLA (rat T-lymphocyte-specific antigens present on T cells of high electrophoretic mobility in thymus and peripheral lymphoid organs). Since the a-RTLA globulin was contaminated with RHLA specificity (14) the presence of RTLA could be tested only in these cells which did not show RHLA.

*F(ab)<sub>2</sub> fragments.* F(ab)<sub>2</sub> fragments of the antiglobulins were prepared and fractionated as described in (15).

*Complement-mediated cytotoxicity.* The complement-mediated cytotoxicity was tested and the cytotoxic index defined as described in detail in (14).

*Antibody-binding capacity.* The antibody-binding capacity was indirectly determined as described by Colley *et al.* (16) modified in (14). The cytotoxic indices were plotted against the number of cells used for absorption in the respective test sample, thus showing an inverse relationship to the absorption capacity of the cells.

*Immunofluorescence.* The IgG fractions and the F(ab)<sub>2</sub> fragments of the antiglobulins were labeled with fluorescein isothiocyanate (FITC Isomere 1) or rhodamine isothiocyanate (TRITC Isomere R, Nordic Pharmaceuticals and Diagnostics) (F/P ratio approximately 4) as described in (17). Excess stain was removed by Sephadex G-25 and the labeled antisera further purified on a Sephadex G-150 column. The antibody titers were adjusted as recently described (14) and viable cells were stained according to Möller (18). The stained cells were examined by incident light in a Zeiss fluorescence microscope.

*Morphology.* The separated cells were suspended in fetal calf serum, smeared on slides, air dried, methanol fixed, and Giemsa stained. Lymphoid cells, normoblasts, and granulopoietic cells were counted at 800-fold magnification.

## RESULTS

### 1. Cell Separations

Viable nucleated rat BM cells show a bimodal sedimentation velocity profile with peaks of varying heights (Fig. 1). The slowly sedimenting peak comprised the small lymphocytes as evaluated on Giemsa-stained smears, and the faster sedimenting cells were granulopoietic cells, larger normoblasts, and other unidentified blast cells (Fig. 1). The small lymphocytes were characterized by means of the surface antigens recently described (14). In all experiments ( $n > 30$ ) more than 80% of the nucleated cells in the slowly sedimenting fractions exhibited the R-Thy-1 antigen

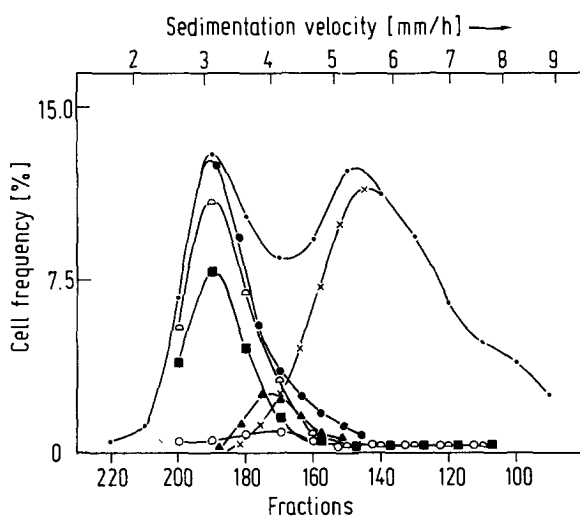


FIG. 1. Typical velocity sedimentation profile of rat bone marrow cells. (·) viable nucleated cells, (●) lymphoid cells, (▲) normoblasts, and (×) granulopoietic cells (evaluated on Giemsa-stained smears). The proportion of cells per fraction showing R-Thy-1 (○), R-Thy-A (■), and RBLA (○) was calculated from the CI.

complex, and about 60% of the cells had R-ThyA antigens. Both sets of antigens were found on all thymocytes but only on a few peripheral lymphocytes (14, 19).

A surprisingly small number of lymphocytes in the unseparated BM as well as in the separated fractions carried RBLA (Fig. 1) which is present on B cells in the spleen and lymph node (14). In a few experiments it seemed that the average sedimentation velocity of the RBLA<sup>+</sup> cells was slightly higher than that of the R-ThyA<sup>+</sup> and R-Thy-1<sup>+</sup> cells. Similarly the number of sIg<sup>+</sup> cells in the starting material, as well as in the separated fractions, was negligibly low. In one experiment the number of sIg<sup>+</sup> cells was increased in the fractions of low to medium sedimentation velocity (Table 1). The small number of sIg<sup>+</sup> cells is unlikely to be due to an experimental artifact since (i) the proportion before and after separation was similar which argues against shedding of Ig from the surface and selective cell loss and (ii) the fluorescein-labeled anti-Ig globulins brightly labeled the surfaces of B lymphocytes in LN and Spl but did not react with thymocytes and peripheral T cells at all (14). The T-cell-specific antigens RTLA and RHLA were found on only a few cells in the slowly sedimenting fractions and were not further analyzed in the sedimentation profile. The slowly sedimenting BM cells (shaded area in inserted profile in Fig. 2) were further separated by free-flow electrophoresis. The resulting profiles showed a narrow, modal, electrophoretic distribution profile with a peak at fraction 50 and a flat skew to the anodic fractions (Fig. 2). Almost all cells in the fractions 48 to 52 were small lymphocytes as evaluated on Giemsa-stained smears (Fig. 3). In the faster fractions about half of the cells were small lymphocytes and the rest were small normoblasts. Within the fractions 48 to 52 about 88% of the cells expressed R-Thy-1 antigens and 66% expressed R-Thy-A antigens, the profiles of which showed narrow modal distributions with peaks in fraction 50 (Fig. 2, Table 2). Double staining of the cells with FITC- and TRITC-labeled a-R-Thy-1 and a-R-ThyA globulin revealed that all cells which exhibit R-ThyA also exhibit R-Thy-1 antigens (Table 2). These cells were not found in the faster fractions.

In the fractions of medium EPM a small modal profile of RBLA<sup>+</sup> cells was observed with a peak at fractions 47 to 48 (Fig. 2). This suggested that the RBLA<sup>+</sup> cells represent a population different from that of the R-Thy-1<sup>+</sup> cells, which was

TABLE 1

Percentage of Surface Ig<sup>+</sup> Cells in Different Fractions of the BM Velocity Sedimentation Profile<sup>a</sup>

Fraction no.	Percentage of surface Ig <sup>+</sup> cells			
	Expt 1	Expt 2	Expt 3	Expt 4
110	2.3	—	—	—
120	2.8	—	—	—
130	1.5	0.98	1.88	1.67
140	3.4	1.02	0.83	1.4
150	5.2	2.48	0.94	0.73
160	12.3	1.03	0.98	1.01
170	13	1.05	2.8	2.31
180	11.6	1.82	2	0.9
190	10.1	1.03	2.01	6.2
200	8.4	0.01	1.88	7.8

<sup>a</sup> The cells were stained with polyvalent rabbit a-rat IgG globulin and FITC-labeled goat a-rabbit IgG globulin. In experiments 3 and 4 F(ab)<sub>2</sub> fragments of the respective antiglobulins were used. See Fig. 1.

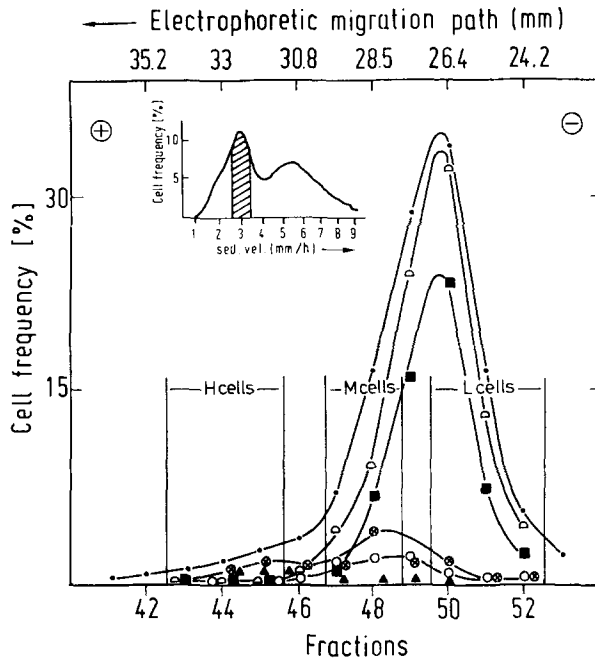


FIG. 2. Electrophoretic distribution profile of rat bone marrow cells sedimenting between 2.5 and 3.5 mm/hr (shaded area in the sedimentation profile). Cells showing different surface antigens were determined by the cytotoxicity test using a-R-Thy-1 ( $\ominus$ ), a-R-Thy-A ( $\blacksquare$ ), a-RBLA ( $\circ$ ), a-RHLA ( $\blacktriangle$ ) globulins. ( $\otimes$ ) Cells showing none of the previous markers, ( $\cdot$ ) viable cells.

also supported by the observation that cells which were stained with FITC-labeled a-RBLA globulin were not counterstained with TRITC-labeled a-R-Thy-1 globulin (Table 2). sIg<sup>+</sup> lymphocytes were observed in only small numbers.

Cells which carried RHLA were found within the fractions 43 to 46 but not at all in the slower fractions (Fig. 2). The same result was observed using an antiglobulin which showed both the a-RTL and a-RHLA specificity. The normoblasts in these fractions did not cross-react with the antilymphocyte globulin as expected from the lack of correlation between the sedimentation profile of normoblasts and RHLA<sup>+</sup> cells. Probably the normoblasts considerably contributed to the bimodal distribution profile of "null" cells in the fractions of medium and high EPM which was obtained by subtraction of the proportion of R-Thy-1<sup>+</sup>, RBLA<sup>+</sup>, and RHLA<sup>+</sup> cells from the nucleated cell profile.

A comparison of the electrophoretic profiles of BM, Thy, and Spl lymphocytes showing discrete surface antigens revealed striking differences. Thymocytes of high and low EPM all carried R-Thy-1, R-Thy-A, and RTL (Fig. 4a). RHLA was found only on thymocytes of high EPM.

In the Spl-lymphocyte profile most of the lymphocytes of low and medium EPM carried RBLA and a large proportion of cells had surface Ig (Fig. 4b). However only negligibly small numbers of cells in these fractions carried R-Thy-1, R-Thy-A, RTL, and RHLA antigens. In the fractions of high EPM nearly all cells were RHLA<sup>+</sup> and considerably less than 5% revealed R-Thy-1, R-Thy-A, RBLA, and sIg. "Null" cells were distributed in the fractions of medium EPM. Most similar electrophoretic distributions of lymphocytes were also observed in the LN (14).

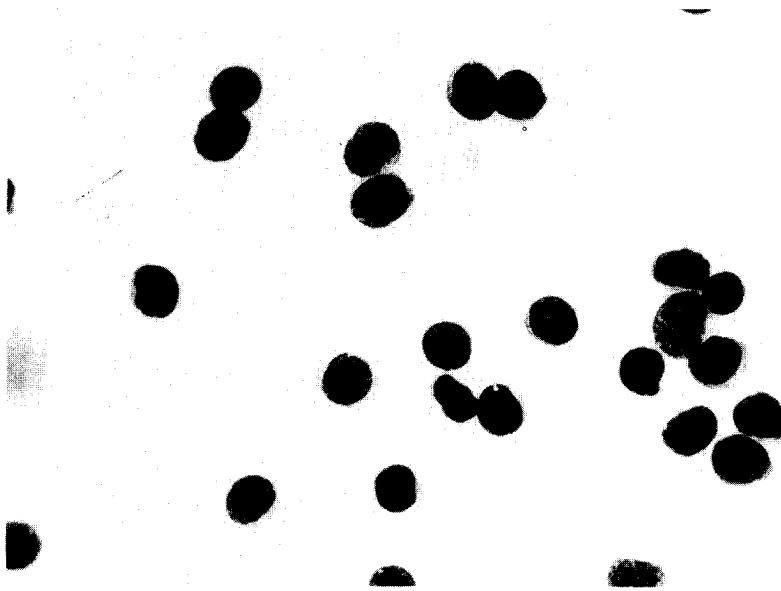


FIG. 3. Photomicrograph of Giemsa-stained bone marrow cells in fraction 50 of the electrophoretic profile shown in Fig. 2.

The only difference was that the fractions of high EPM contained more than 5% R-Thy-1<sup>+</sup> cells (19).

## 2. Absorption Capacities

The distribution of surface antigens on separated lymphocytes was further analyzed measuring the relative antibody-binding capacity. The cells in the electrophoretic profiles were pooled within a range of high EPM (H-cells), medium EPM (M-cells), and low EPM (L-cells) which contain the various discrete cell populations observed (Figs. 2, 4a,b). Graded numbers of the pooled cells were

TABLE 2  
Surface Antigens on Electrophoretically Separated BM Lymphocytes Sedimenting  
between 2.5 and 3.5 mm/h<sup>a</sup>

Percentage bone marrow lymphocytes stained by direct immunofluorescence							
Fraction no.	(RTL/RHLA) <sup>+</sup>	RThy-1 <sup>++</sup>	RThyA <sup>+</sup>	RBLA <sup>+</sup>	Ig <sup>+</sup>	RThy-1 <sup>++</sup> RThyA <sup>+</sup>	RThy-1 <sup>++</sup> RBLA <sup>+</sup>
44	57	—	—	—	1.4	—	—
45	58	—	—	4	2.1	—	—
46	41	2	—	7	1.9	—	—
47	13	2	—	12	1.3	1	—
48	5	36	29	9	2.2	29	0.05
49	2	57	44	4	4.2	43	0.09
50	—	85	62	5	3.2	62	—
51	—	95	73	3	4.3	73	—
52	—	93	66	1	—	66	—

<sup>a</sup> The cells were stained with FITC<sup>(+)</sup>- or TRITC<sup>(++)</sup>-labeled rabbit a-rat lymphocyte globulins.

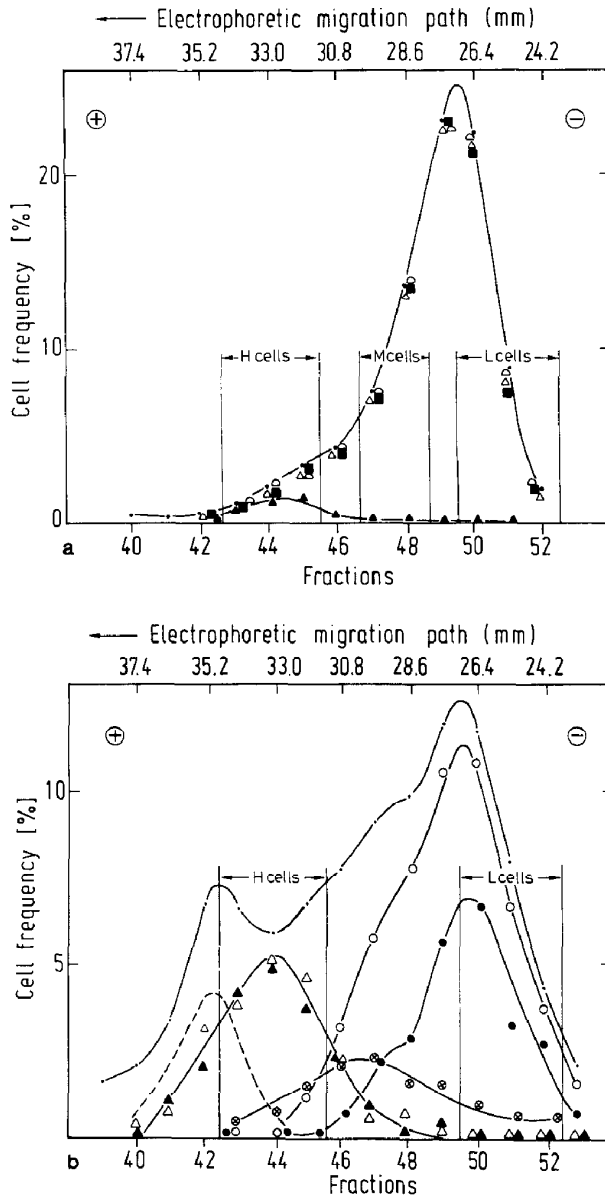


FIG. 4. Electrophoretic distribution profile of rat thymocytes (a) and spleen cells (b). (·) total cells; (---) erythrocytes. Cells showing different surface antigens were determined by the cytotoxicity test using a-R-Thy-1 (⊖), a-R-Thy-A (■), a-RBLA (○), a-RHLA (▲), and a-RTL/RHLA (Δ) globulin. (●) Surface Ig<sup>+</sup> cells were determined using polyvalent rabbit a-rat IgG globulin and FITC-labeled goat a-rabbit IgG globulin. (⊗) Cells showing none of the previous markers.

incubated with standard amounts of antiglobulins and the complement-mediated cytotoxicity of the supernatant tested with antigen-specific target cells. Figure 5 shows that Spl-L cells and, to a considerably less degree BM-M cells, absorbed a-RBLA specificity, whereas the binding capacity of Spl-H cells, BM-H cells, and BM-L cells was negligible. Thymocytes also did not react with a-RBLA globulin (14). These results are in good agreement with the distribution of RBLA<sup>+</sup> cells in the tested samples.

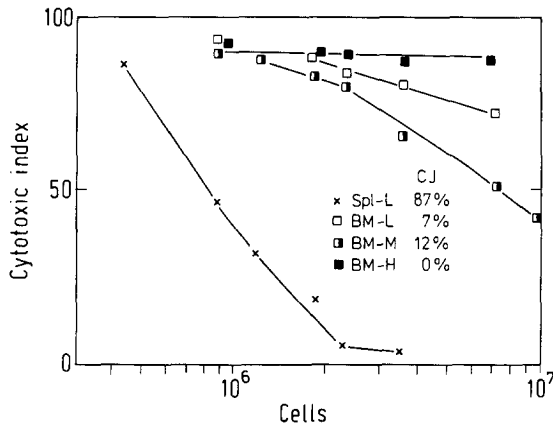


FIG. 5. Cytotoxic activity of rabbit a-RBLA globulin against Spl-L target cells after absorption with graded numbers of electrophoretically separated Spl-L cells (x), BM-L cells (□), BM-M cells (◻), and BM-H cells (■). The BM cells had been previously separated by means of the 1-g sedimentation and had been pooled within 2.5 to 3.5 mm/hr. The proportion of cells carrying the respective antigens in the samples to be tested was determined by the CI.

For the analysis of the RHLA and RTLA specificity an antiglobulin was used which contained either RHLA activity or both the RHLA and the RTLA activity. These specificities were specifically removed by BM-H cells but not by BM-L cells (Fig. 6). On the other hand thymocytes of all different EPM bound a-RTLA globulin whilst a-RHLA activity was only absorbed by H-cells. In Spl and LN both specificities were absorbed by H-cells but not by L-cells (14). Thus in this case, too, the distribution of RHLA<sup>+</sup> and RTLA<sup>+</sup> cells in the electrophoretic profiles is in good agreement with the absorption tests.

Similarly the a-Thy-1 specificity, including the subspecificities a-R<sub>s</sub>-Thy-1 and a-R-Thy-1.1, was significantly absorbed by BM-L cells and thymocytes of all different EPM but not by BM-H cells and Spl cells (Figs. 7a-c). Since in the BM-L

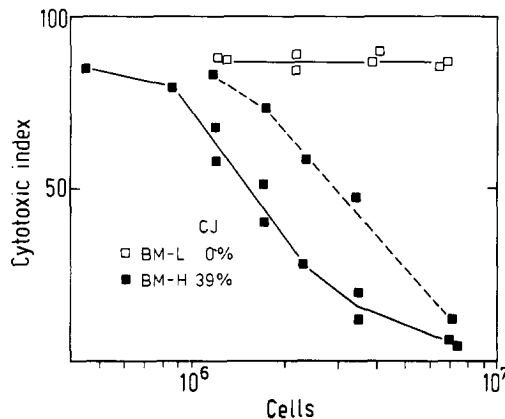


FIG. 6. Cytotoxic activity of rabbit a-RHLA (---) and a-RTLA/RHLA (—) globulin against Spl-H target cells after absorption with graded numbers of electrophoretically separated BM-L cells (□) and BM-H cells (■). The BM cells had been previously separated by means of the 1-g sedimentation and pooled within 2.5 to 3.5 mm/hr. Different experiments are shown. The proportions of cells carrying the respective antigens in the samples to be tested were determined by the CI.



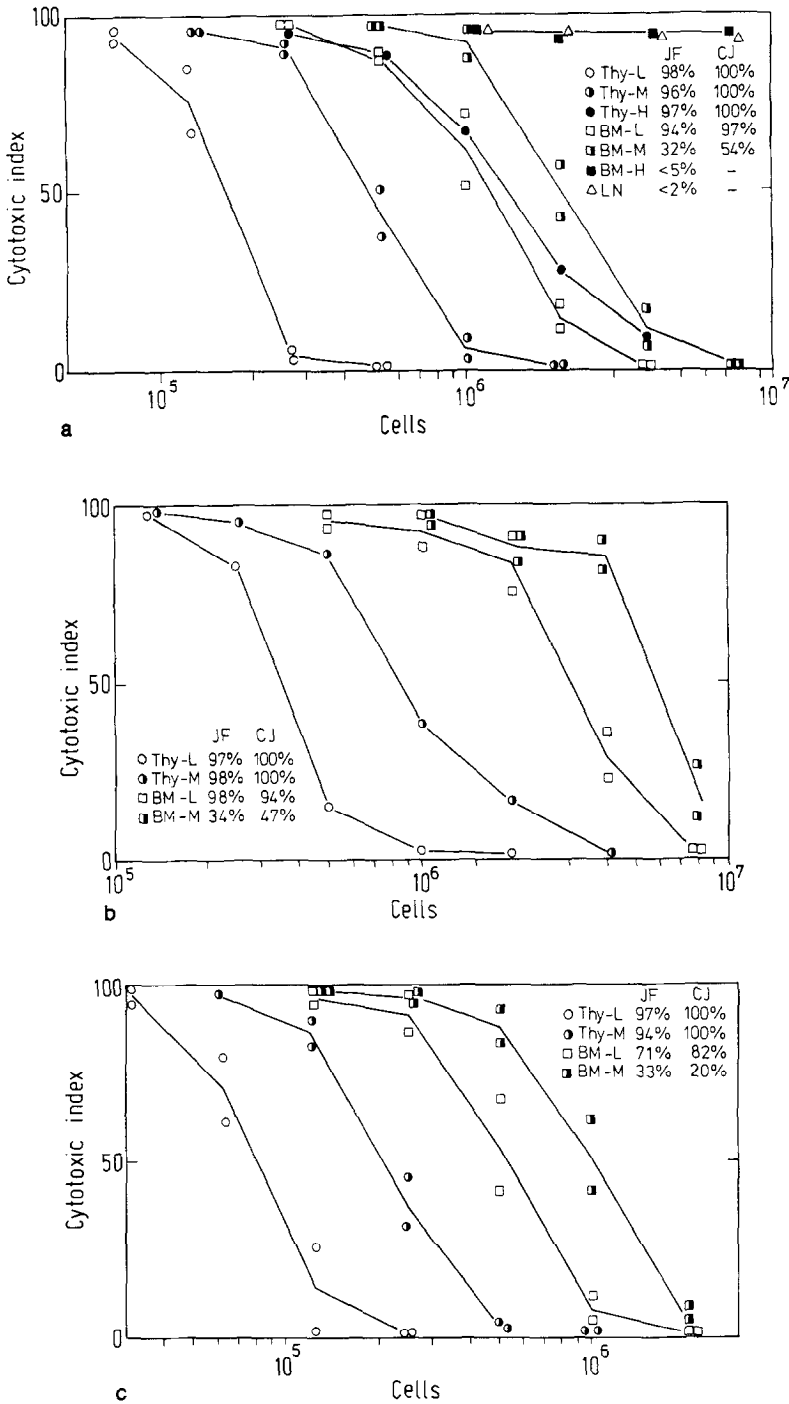


FIG. 7. Cytotoxic activity of rabbit a-RThy-1 (a), a-RThy-1.1 (b) and a-R<sub>s</sub>-Thy-1 (c) globulin against thymocytes after absorption with graded numbers of electrophoretically separated Thy-L cells (○), Thy-M cells (●), Thy-H cells (●), BM-L cells (□), BM-M cells (■), BM-H cells (■), and unseparated LN cells (△). The BM cells had been previously separated by means of the 1-g sedimentation and had been pooled within 2.5 to 3.5 mm/hr. Different experiments are shown. The proportion of cells carrying the respective antigens in the samples to be tested was evaluated by the CI and indirect immunofluorescence (IF) using rabbit a-rat globulin and FITC-labeled goat a-rabbit IgG globulin.

TABLE 3

Comparison of the Relative Absorption Capacity of Electrophoretically Separated BM and Thy Cells for a-RThy-1, a-R<sub>s</sub>Thy-1, a-RThy-1.1, and a-RThy-A Globulin<sup>a</sup>

Cells	Relative number of cells which absorb 50% cytotoxicity			
	a-RThy-1	a-R <sub>s</sub> Thy-1	a-RThy-1.1	a-RThyA
BM-L	7.1	6.1	8.6	12
Thy-H	8.2	n.d.	n.d.	1
Thy-M	2.9	2.7	2.3	1
Thy-L	1	1	1	1

<sup>a</sup> See Fig. 7a-c.

cell pool and the different thymocyte pools almost all cells were Thy-1<sup>+</sup> and showed similar size variations (10), the average relative binding capacity per cell-surface unit area may be roughly estimated at the 50% cytotoxicity level. A comparison of Figs. 7a-c shows that the a-R-Thy-1, a-R<sub>s</sub>-Thy-1, and a-Thy-1.1 specificities were absorbed at similar rates by the same cell pools, but were absorbed at significantly different rates by the different cell pools (Table 3). It appeared that BM-L cells and Thy-H cells required about seven- to eightfold more cells to remove 50% cytotoxicity from the antiglobulins than Thy-L cells, whilst only about two- to threefold more Thy-M cells were necessary to obtain the same result. These differences could be confirmed measuring Thy-1- surface-antigen densities with a fluorescence-activated sorter (19). Moreover we observed that BM-L cells exhibited 20% more Thy-1 antigen than Thy-H cells. Thus it seems that Thy-1<sup>+</sup> BM lymphocytes are different from all thymocytes. This also accounts for the surface density of R-Thy-A which was fairly similar on the thymocytes of different EPM but was significantly lower on BM-L lymphocytes (Fig. 8, Table 3).

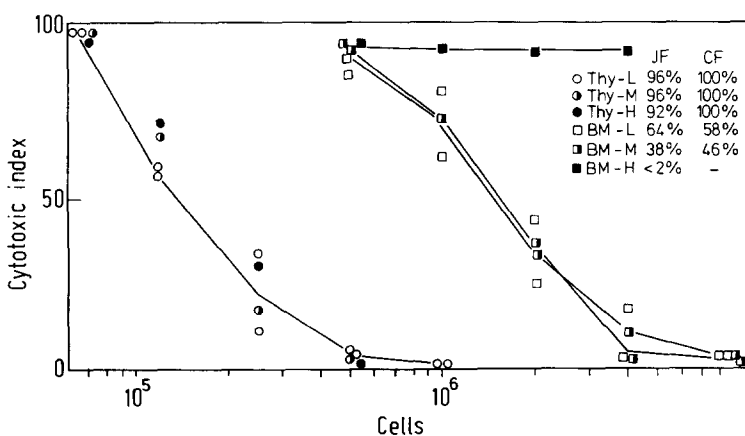


FIG. 8. Cytotoxic activity of rabbit a/RThy-A immunoglobulin against thymocytes after absorption with graded numbers of electrophoretically separated Thy-L cells (○), Thy-M cells (◐), Thy-H cells (●), BM-L cells (□), BM-M cells (◑), and BM-H cells (■). Different experiments are shown. The BM cells had been previously separated by means of 1-g sedimentation and had been pooled within 2.5 to 3.5 mm/hr. The proportion of cells carrying the respective antigens in the samples to be tested was evaluated by the CI and indirect immunofluorescence (IF) using rabbit a-rat immunoglobulin and FITC-labeled goat a-rabbit IgG immunoglobulin.

### 3. Cortisone Sensitivity

The small BM lymphocytes of low EPM were considerably resistant to cortisone. This was suggested by the observation that prednisolone doses which nearly eradicated small thymocytes of low EPM (10) hardly affected the velocity sedimentation and the electrophoretic distribution profiles of the BM cells.

## DISCUSSION

Nucleated bone marrow cells with a volume between 90 and 120  $\mu\text{m}^3$  were isolated by velocity sedimentation, further separated by free-flow electrophoresis, and characterized by lymphocyte-specific antisera which have been recently described (14). More than 85% of these cells were lymphocytes as evaluated by their morphology and reactivity with the lymphocyte-specific antisera. About half of the cells of high EPM (which represent a negligibly small number of all BM cells) carried RHLA which is a specific marker for mature T cells in the peripheral lymphoid organs (14). Since thymocytes specific and B-cell-specific markers were not detected on these cells it may well be that they represent recirculating T cells. The fractions of medium and low EPM contained a few lymphocytes showing the B-cell markers RBLA and surface Ig. This is in marked contrast to the LN and Spl where most of the lymphocytes of medium and low EPM are RBLA<sup>+</sup> and sIg<sup>+</sup> (14). In the fractions of low EPM almost all of the cells (which comprise more than 80% of the small BM lymphocytes) carried all subspecificities of the rat Thy-1 antigen complex (21) and a large number of these cells also expressed R-Thy-A which is a rat thymus-specific marker (14). Another outstanding finding was that the Thy-1<sup>+</sup> BM cells lack RTLA and RHLA, which are specific T-cell markers in the thymus and periphery (14), and lack the B-cell markers RBLA and sIg.

The surface densities showed characteristic differences in cell populations from different lymphoid organs. The density of R-Thy-A was several-fold lower on BM lymphocytes than on all thymocytes. In agreement with recent results (6, 8, 22) the R-Thy-1 antigen complex was found at considerably higher densities on the bulk of thymocytes which show low and medium EPM than on BM lymphocytes. On the other hand the BM lymphocytes revealed higher densities of R-Thy-1 antigens than thymocytes of high EPM. These results could be confirmed using a fluorescence-activated flow sorter for measuring surface-antigen densities (19). In the peripheral lymphoid organs Thy-1<sup>+</sup> cells were observed only in the LN and showed high EPM and surface densities similar to the thymocytes of high EPM (19). A further difference between the Thy-1<sup>+</sup> BM cells and the thymocytes of low EPM is the relative cortisone resistance of the former cells. Thus most of the rat BM lymphocytes are clearly different from all thymocytes and lymphocytes in the peripheral lymphoid organs.

These results fit with Goldschneider's (7) observations which showed that 80% of the rat BM lymphocytes do not react with antibodies specific for mature T and B cells but are readily labeled with an anti-BM-lymphocyte antiserum which cross-reacted with cortisone-sensitive lymphocytes in the thymus, blood, and spleen. Our failure to detect sIg on the Thy-1<sup>+</sup> BM lymphocytes disagrees with the observations of Hunt *et al.* (9) who found that about 25% of these cells carry endogenous sIg. It is unlikely that this discrepancy is due to experimental error since our rat thymocyte-absorbed polyvalent rabbit a-rat Ig globulin was B-cell specific (14) and even a sensitive technique such as a fluorescence-activated flow

sorter failed to detect sIg on unseparated as well as separated Thy-1<sup>+</sup> BM lymphocytes of low EPM (E. Hansen, L. Voet, and K. Zeiller, unpublished data). It seems more likely that Hunt's a-Ig antisera possess a cross-reacting specificity which is absent in our antisera. This explanation is supported by the work of Santana *et al.* (23, 24) who showed that some rabbit antisera raised against mouse Ig, light chains, or  $\mu$  chains cross-reacted with thymocytes whilst others did not. It appeared that the cross-reacting thymocyte-surface component was a high-molecular-weight compound, which was not Ig in nature.

The functional potential of rat Thy-1<sup>+</sup> BM lymphocytes remains unclear. Hunt *et al.* (9) suggested that the bulk of the Thy-1<sup>+</sup> BM lymphocytes represents an intermediate stage in the B-cell development. They observed that Thy-1<sup>-</sup>, sIg<sup>+</sup>B cells develop from Thy-1<sup>+</sup>, sIg<sup>-</sup>-precursor cells in the marrow and argued that the Thy-1 antigen is lost by the early B cells when they leave the marrow. This view has some support from Goldschneider's finding that lymphopoietic stem cells share antigens with BM lymphocytes and thymocytes (25). However, there is no direct evidence that the bulk of BM lymphocytes of low EPM which show Thy-1 antigens are a progeny of these B-cell precursors. On the contrary there is more evidence against than for a B-cell relationship: (i) The separated rat small BM lymphocytes (in contrast to unseparated BM cells) provide a mitogenic response to the T-cell mitogen Con A which is not shown by the B lymphocytes in LN and Spl (26). (ii) In the marrow of nu/nu mice very similar cells were found which are certainly not B cells. They are small noncirculating lymphocytes of low EPM, which lack sIg and MBLA but carry low densities of Thy-1 antigens and TL antigens (27, 28). These cells are born in the marrow and seem to be under some homeostatic influence of the thymus (27). In the normal mouse these cells are hardly detectable. Almost all lymphocytes of low EPM in the marrow and peripheral lymphoid organs of normal mice represent mature B lymphocytes which are Thy-1 negative and carry high densities of MBLA and sIg (29). Recently it was shown that these cells are not self-renewing but are provided by precursor cells of medium and high EPM which express low densities of sIg and MBLA (30). It appears that the decrease in EPM is a late event in the maturation of B cells which follows, rather than precedes, the expression of sIg and MBLA (30, 31). Considering the close similarity in the electrokinetic behavior of functionally corresponding lymphocytes in rat and mouse (32) there is no reason to assume that the Thy-1<sup>+</sup> rat BM lymphocytes of low EPM represent early B cells which lack sIg and RBLA keeping in mind that almost all peripheral rat lymphocytes of low EPM carry RBLA and sIg (29) and that the marrow contains a few cells of medium EPM which carry these markers too.

It may be that these Thy-1<sup>+</sup> rat and mouse BM lymphocytes of low EPM belong to the T-cell lineage. This is supported by the observation that treatment of nu/nu mice with ubiquitin or thymopoietin increased the number of these cells in the marrow (33) and that they responded to Con A and MLC after culturing on reticuloepithelial cells (34). Thus it may be that these Thy-1<sup>+</sup> BM lymphocytes of low EPM are pre-T cells as suggested by various authors (6, 7, 27, 28, 33, 34). Nevertheless their role in the T-cell lineage is still speculative. In thymusless mice they are produced in relatively high numbers in the marrow, emigrate to the periphery, but do not give rise to mature T cells of known function (27, 28, 34). It seems that the thymus is required for the differentiation of the Thy-1<sup>+</sup> BM lymphocytes of low EPM supposing they undergo any further differentiation into functional T cells. This influence may act indirectly via a secreted hormone or

directly on immigrant cells. The fact that these cells were not detected in the thymus does not invalidate the latter argument. Recently it was observed that only a few cells are required to repopulate an irradiated thymus (35, 36) and we may assume that even less immigrant cells are needed to replace the differentiating thymocytes in a normal thymus. Thus newly immigrant prothymocytes may be present in numbers too small to be detected and the cell surface of immigrant cells may also change rapidly. However, prothymocytes have been described as medium- to large-sized cells whilst the Thy-1<sup>+</sup> BM lymphocytes of low EPM are small sized (10, 35). Taking these various facts into consideration it is clear that considerably more information is required before the role of these cells in the T- and B-cell system can be precisely classified.

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