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J J Twomey, … , R J Luchi, N M Kouttab

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Research Article

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Null Cell Senescence and Its Potential Significance to the Immunobiology of Aging

JEREMIAH J. TWOMEY, ROBERT J. LUCHI, and NICOLA M. KOUTTAB,
Immunohematology Research Laboratory, Veterans Administration Medical Center, Houston, Texas 77211; Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

ABSTRACT The null cell compartments of human bone marrow and mouse spleen were arbitrarily divided into three subpopulations based upon the ability of cells to acquire T or B cell membrane markers when incubated with poly A:U or ubiquitin. There was an accumulation of T cell precursors with congenital absence of the thymus. In contrast, T cell precursors were reduced and there was an accumulation of uninduced null cells with old age. These observations suggest that there is an intrinsic defect of null cell differentiation with a drift towards more differentiated precursors in T cell differentiation with aging. This could result in a diminution in the range of responses by their progeny, mature T lymphocytes.

INTRODUCTION

Immune responsiveness decreases with advanced age (1–3). This decline is accompanied by reduced longevity. For example, the probability of surviving 2 yr is significantly reduced among elderly subjects, if they are anergic (4). Immunologic senescence affects T lymphocyte function most severely (5, 6).

Immature precursor cells first differentiate into null lymphocytes. Many null lymphoid cells are committed to T or B cell differentiation. We arbitrarily divide the null cell compartment into three subpopulations based upon acquisition of membrane markers when incubated with the inducing substances ubiquitin (7) or poly A:U. Those that acquire T cell markers are called pre-T cells; those that acquire B cell markers pre-B cells, and those that acquire neither, uninduced null cells. The composition of the latter is probably heterogeneous.

This study compares the impact of aging and congenital absence of the thymus upon the null cell compartment. The changes observed with senescence clearly differ from those associated with a primary athymic state, and probably contribute to the immunobiology of aging.

METHODS

We studied five healthy volunteers aged 20–30 yr and four subjects >75 yr old who resided in a nursing home because of age-related management problems and were without known immunologic disease. Heparinized bone marrow was aspirated in <0.5-ml aliquots and mononuclear cells harvested by isopyknic flotation. Spleen cell suspensions were obtained from C3H/He conventional mice ($H\text{-}2^k$) at ages 3 and 24 mo, and germ-free, nude, congenitally athymic mice at age 3 mo. The nude mice were bred on a C3H and Swiss background ($H\text{-}2^{b,k,p}$).

The protocol for examining the null cell compartment has been described (7). Briefly, 0.5×10^6 cells in 0.2 ml Medium 199 (Microbiological Associates, Bethesda, MD) and 5% bovine serum albumin were incubated at 37°C for 18 h with and without inducing agent. Induction of T or B cell phenotypes was with 2.5 μg/ml poly A:U (Sigma Chemical Co., St. Louis MO) with human bone marrow and 5 ng/ml ubiquitin with mouse spleen cells (8). T cells were identified using a monoclonal antibody to Thy 1.2 antigen (New England Nuclear, Boston, MA) in mouse experiments and OKT3 monoclonal antibody (Ortho Pharmaceutical, Raritan, NJ) in human experiments. B lymphocytes were identified by the presence of surface membrane immunoglobulins (Ig) in mouse experiments and using OKIa monoclonal antibody (Ortho Pharmaceutical) in human experiments. Similar data was obtained when human T cells were identified by E rosette formation or membrane T_{11} antigen (Coulter Electronics Inc., Hialeah, FL), and pre-B cells by C3b membrane receptors or B-I antigen (Coulter Electronics). Cells with lymphoid morphology that reacted with these antibodies were enumerated by indirect UV microscopy on counts of 300 cells; similar data was obtained using a cytofluorograf (Ortho Pharmaceutical) (7). Lymphocyte counts were performed on Wright-Giemsa-stained smears.

Pre-T cell percentages were determined by subtracting Thy 1.2^+ or $OKT3^+$ cells in uninduced incubations from similar counts done on paired induced incubations. Pre-B cells were calculated similarly from differences in membrane Ig^+ or $OKIa^+$ cells in paired induced and not induced incubations. Uninduced cells represent the difference between total lymphocyte and T plus B cell counts on induced incubations.

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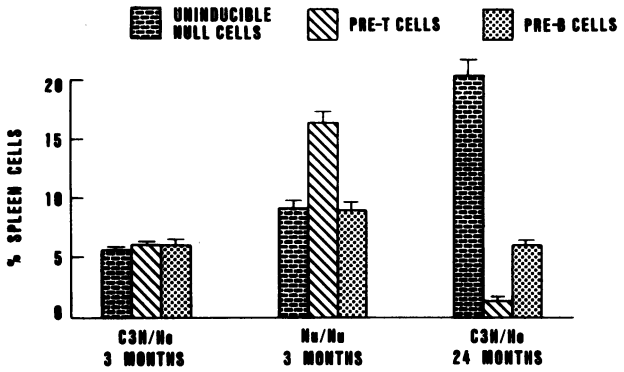

FIGURE 1 The subpopulations that comprise the null cell compartment of 3-mo-old healthy mice, 3-mo-old congenitally athymic (Nu/Nu) mice, and aged mice born with a normal thymus.

RESULTS

10 conventional mice were studied at age 3 mo. Spleen cell suspensions included 5.5±0.2% uninduced null cells, 6.0±0.2% null cells that were induced to express Thy 1.2 antigen (pre-T cells), and 6.0±0.4% null cells that were induced to express membrane Ig (pre-B cells) when incubated with ubiquitin (Fig. 1). Similar data were derived from absolute cell counts. Eight 3-mo-old athymic mice were studied. When adjustments were made for the absence of T cells in these spleens, the uninduced null cell and pre-B cell content did not differ significantly from age-matched controls ($P > 0.05$). However, pre-T cells were significantly elevated (17±1%) ($P < 0.01$). Six conventional mice were studied at age 24 mo. Their spleens did not differ significantly from those of 3-mo-old mice in absolute or percent T lymphocyte, B lymphocyte, or pre-B cell content. In contrast, these aged spleens had only 1.1±0.1% pre-T cells ($P < 0.01$) but had 20.5±1.3% uninduced null cells ($P < 0.01$).

Similar changes were observed with aging in the null cell compartment of human bone marrow. The number of pre-T cells was greatly reduced (<0.025) and uninduced null cells were 8.7-fold more numerous than in marrow from young adults ($P < 0.001$) (Table I). There was no significant difference in the numbers of T lymphocytes, B lymphocytes, or pre-B cells per million bone marrow lymphocytes from young and aged subjects.

DISCUSSION

The null cell compartment of the mouse spleen or human bone marrow includes three defined subpopulations based upon the appearance of T or B lymphocyte markers when incubated with ubiquitin or poly A:U. Similar changes were observed in both species with advanced age. These changes include a decrease in pre-T null cells and an accumulation of uninduced null cells. The apparent exclusion of B cell precursors from null cell senescence may have bearing upon the relative preservation of B cell function into old age (5, 6).

The thymus has an important role in the differentiation of T lymphocytes. The thymus undergoes severe structural involution and no longer secretes thymic hormone in man by the seventh age decade (9). This prolonged state of thymic deprivation is felt to contribute to the immunobiology of aging (10). It is evident from this study that changes in lymphocyte precursors with advanced age cannot be ascribed directly to thymic involution. An accumulation of T cell precursors was observed in 3-mo-old mice who were congenitally athymic. Conversely, mice born with a normal thymus had reduced numbers of pre-T cells but a marked accumulation of uninduced null cells when studied at an advanced age. Perhaps, with advanced age, null T lymphocyte precursors become unresponsive to inductive stimuli. Aged mouse null cells are unresponsive to the thymic peptide thymopoietin, as well as to ubiquitin (7).

Total T lymphocyte mass is sustained into old age (5, 11). The present study shows that this is accomplished despite reduced replacement of T lymphocytes from the null cell compartment. This suggests that T cells in the elderly are largely derived from more differentiated precursors that already express Thy 1.2 antigen in the mouse and OKT3 antigen in man.

These conclusions suggest a new hypothesis: mature T lymphocyte clones are normally derived from replication and maturation of null cells and early T cells (Fig. 2). In later life, more T lymphocytes are derived from early T cells because of reduced input from the less differentiated null cell compartment. Cell differentiation is accompanied by a reduced potential for functional diversity. This restriction attains clonal specificity at T cell maturity. We propose that this shift toward greater maturity in T cell precursors with aging narrows the range of responses that their progeny can undertake.

TABLE I
Changes Observed in the Composition of the Null Cell Compartment in Human Bone Marrow Mononuclear Cells with Aging

Age	T cells	B cells	Pre-T cells	Pre-B cells	Uninducible null cells
	% of bone marrow lymphocytes				
20–30 yr	42±4	27±4	16±3	14±3	3±1
>75 yr	33±2	21±2	4±2	17±2	26±1
P	NS	NS	<0.025	NS	<0.001

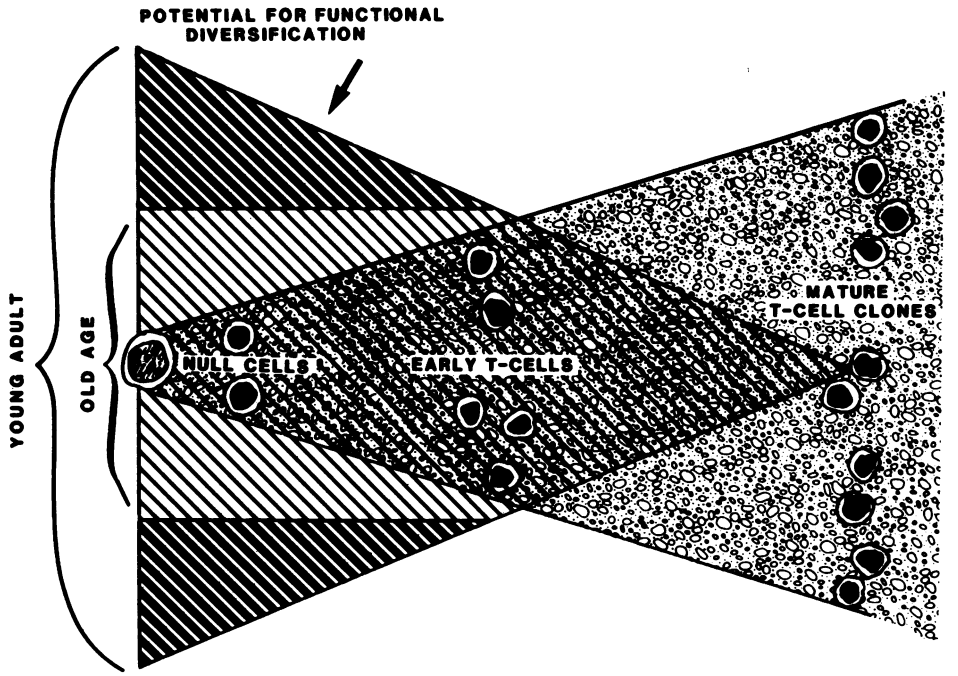

FIGURE 2 A schematic outline of T cell differentiation and how a shift to the right in T cell precursors could restrict the range of T cell-dependent immune responses.

There is evidence to support the hypothesis that the range of immune responses becomes restricted with aging. Antigens usually evoke a number of antibody responses with variable affinity for antigen binding. There is a restriction in the range of antigen avidity by T cell-dependent antibody responses with aging (12, 13). Proliferative responses by T lymphocytes to polyclonal mitogens were 44% less with mononuclear cells from elderly than from younger donors, but were 67% less to soluble antigen, known to stimulate considerably fewer T cells (14).

The immunobiology of aging is a multifactorial problem. Thymic involution is probably involved (10). Effector T lymphocytes become intrinsically defective (15). These cells are more easily inhibited by suppressor T lymphocytes (16) or monocytes (17). Suppression by both T lymphocytes (18) and monocytes (19) is also increased. The proposed additional mechanism, involving functional restriction because of null cell senescence should be considered within the framework of this more global concept.

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