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Age-related change of distribution of immunoglobulin containing cells in human bone marrow

Changes in patients with benign monoclonal gammopathy and multiple myeloma

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Summary. The number and mode of distribution of immunoglobulin containing cells in human bone marrow were investigated immunohistochemically using paraffin sections of bone marrow aspirates. In individuals without specific diseases, the number of immunoglobulin containing cells per unit field in bone marrow increased with advancing age until the 3rd decade and leveled off thereafter. The magnitude of the increase was great for Ig-G and Ig-A, but very slight for Ig-M. Such age-related change in the number of Ig-G and Ig-M containing cells in bone marrow was almost comparable to the age-related change of serum level of Ig-G and Ig-M. However, the magnitude of age-related increase of Ig-A containing cells in bone marrow was apparently higher than that of the serum level of Ig-A. Cluster formation of immunoglobulin containing cells increased with age in terms of both incidence and size. Three points were suggested for differentiation of benign monoclonal gammopathy (BMG) from multiple myeloma (MM). First, the ratio of serum level of M-component divided by the average number of immunoglobulin containing cells per unit field was higher in BMG than in MM; second, the number of cells per cluster of immunoglobulin containing cells was more than 50 in MM, but that in BMG less than 20; third, the small immunoglobulin containing cells with narrow cytoplasm were more prominent in MM than in BMG.

Key words: Immunoglobulin containing cells – Human bone marrow –Aging – Benign monoclonal gammopathy – Multiple myeloma

The immune system can respond to various kinds of antigenic stimulation, showing proliferation of a spectrum of immune cell clones corresponding to the antigens and resulting in either a humoral immune response by producing

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immunoglobulins, or a cellular immune response by proliferation of T cells. The immune response occurs mainly in the peripheral lymphoid tissues such as spleen, lymph nodes, tonsils and lymphoid tissues associated with the digestive and respiratory tracts. Actually, immunoglobulin containing cells can be easily detected immunohistochemically in routine paraffin sections of these peripheral lymphoid tissues.

In contrast, the bone marrow, the total mass of which is about 4.0–5.0% of body weight and almost comparable to that of the liver (Erslev 1967), has been generally regarded as the source of haemopoietic cells such as leukocytes, red blood cells and platelets, and its immunological aspect has been estimated mainly in terms of source of the progenitors of T and B cells. Recently the bone marrow has been reappraised as the major source of immunoglobulins (Benner et al. 1981) and in aged mice the major site of antibody production is known to move from the spleen to bone marrow (Haaijman and Hijmans 1978). Serum levels of immunoglobulins were reported to increase with age in man (Buckley and Dorsey 1970; Kishimoto et al. (1978) in association with increased incidence of monoclonal M components (Axelsson et al. 1966). Consequently, incidence of benign monoclonal gammopathy (BMG) and multiple myeloma (MM) is known to increase with advance in age (Radl et al. 1975). These facts suggested that bone marrow is not only the source of progenitors of immunoglobulin producing cells, but that it may also be an important production site of immunoglobulins.

Thus, in the present paper, an immunohistochemical study was performed to determine the age-related changes of human bone marrow without specific diseases in terms of the number and mode of distribution of immunoglobulin containing cells. The results were compared with those in benign monoclonal gammopathy and multiple myeloma.

Materials and methods

Bone marrow aspiration

Aspirated bone marrows were used in the present study. They were obtained at the Japan Red Cross Medical Center and Nakano General Hospital. Since bone marrow specimens were obtained with difficulty from healthy people, bone marrows obtained from patients with the following criteria were used as controls for aging individuals: 1, No malignancy; 2, serum level of Immunoglobulin within normal limits; 3, no M-component in the serum; 4, no immunosuppressive treatment. For assessing age-related changes, 7 to 11 cases were selcted in each decade and a total of 92 cases were examined. Clinically, they were composed of idiophatic thrombocytopaenic purpura (18), iron deficiency anaemia (15), aplastic anaemia of mild type (6), acute infection (6), megaloblastic anaemia (5), rheumatoid arthritis (5), leukocytosis (4), leukopaenia (4), neurological diseases (4) and others (25) (Table 1). Aspirated bone marrow from 6 cases of benign monoclonal gammopathy of Ig-G type and 7 cases of multiple myeloma of Ig-G type were also examined. All specimens were obtained from patients before treatment.

Indirect fluorescent antibody method

Aspirated bone marrow specimens were fixed with 4% buffered formaldehyde (ph 7.2) and processed for paraffin sectioning as routinely performed. Paraffin sections of 6 μ were deparaf-

Table 1. Age and number of examined cases

AGE	Control	Benign monoclonal gammopathy of Ig-G type	Multiple myeloma of Ig-G type
0– 1	5(2/3)		
2– 4	7(4/3)		
5– 9	7(4/3)		
10–19	8(4/4)		
20–29	9(4/5)		
30–39	10(4/6)	1(0/1)	
40–49	9(5/4)		
50–59	9(3/6)	1(1/0)	2(1/1)
60–69	10(6/4)	3(0/3)	4(2/2)
70–79	11(5/6)	1(1/0)	1(1/0)
80–	7(4/3)		
Total	92(45/47)	6(2/4)	7(4/3)

Note: Number in parentheses indicate ratio of male/female

finized in xylene, hydrated through graded ethanol and brought to water. The sections were washed in phosphate buffered saline (PBS, ph 7.2) and treated with 0.25% trypsin (DIFCO Lab. USA) in PBS at 37° C for 30 min. They were then washed in PBS, masked with normal goat serum (1:7 dilution) to remove non-specific staining, reacted with primary antiserum for 30 min at room temperature and washed again in PBS. They were then reacted with FITC conjugated secondary antiserum for 30 min at room temperature, washed in PBS and mounted in buffered 90% glycerin (pH 7.2).

The primary antisera were rabbit anti-human Ig-G, Ig-M or Ig-A (Behring Institute, West Germany) and used at dilution of 1:80. The secondary anti-serum was FITC-conjugated goat anti-rabbit Ig-G (Behring Institute. West Germany) and used at dilution of 1:20. The sections were examined by fluorescent microscope (Nikon, Biophoto) and the number of positive cells in a field was counted at magnification of × 1000. The sum total number of positive cells in four randomly selected places was defined as the number of positive cells per unit field. Patterns of the distribution of positive cells were examined at magnification of × 200 and × 400.

The level of serum immunoglobulin was assessed according to the method of single radial immunodiffusion.

Results

Age-related changes in immunoglobulin containing cells in the bone marrow

The number of Ig-G containing cells per unit field in the control group showed a rapid increase until the 3rd decade and a gradual increase thereafter until the 9th decade. The number of Ig-A containing cells showed also a rapid increase during the 1st decade, a moderate increase thereafter until the 6th decade and leveled off after the 7th decade. The number of Ig-M containing cells showed a slight increase until the 3rd decade and leveled off thereafter (Fig. 1).

The patterns of age-related change in the number of Ig-G and Ig-M containing cells in bone marrow were quite comparable to those of age-related change in the serum level of Ig-G and Ig-M (Fig. 2). However, the

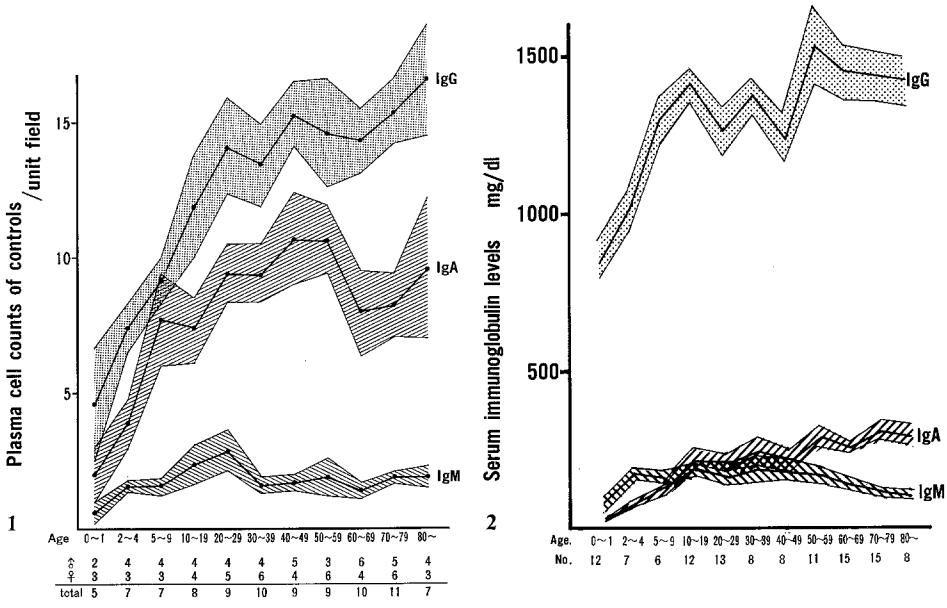

Fig. 1. Age-related change in the number of immunoglobulin containing cells per unit field in human bone marrow. Solid circles, bound with continuous lines indicate the mean values of each age group and shaded areas, one standard error of the mean. In abscissa, age groups with the number of male and female individuals were indicated

Fig. 2. Age-related change of serum immunoglobulin levels (mg/dl). Continous lines indicate mean values and shaded areas, one standard error of the mean. Age groups with the number of individuals examined are indicated in the abscissa

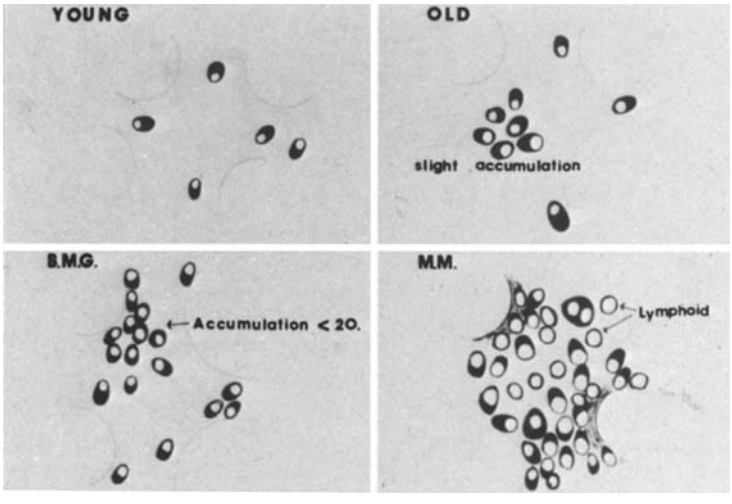

Fig. 3. Schematic presentation of distribution pattern of Ig-G containing cells in bone marrow of young, old, benign monoclonal gammopathy (*BMG*) and multiple myeloma (*MM*). Cluster of immunoglobulin containing cells increases in number and in size with advance of age. The number of immunoglobulin containing cells per cluster is less than 20 in BMG and more than 50 in MM

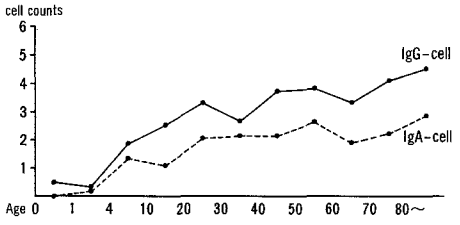

Fig. 4. Age-related change in cell count in a cluster of immunoglobulin containing cells in human bone marrow. Each point indicates average of 5–11 samples

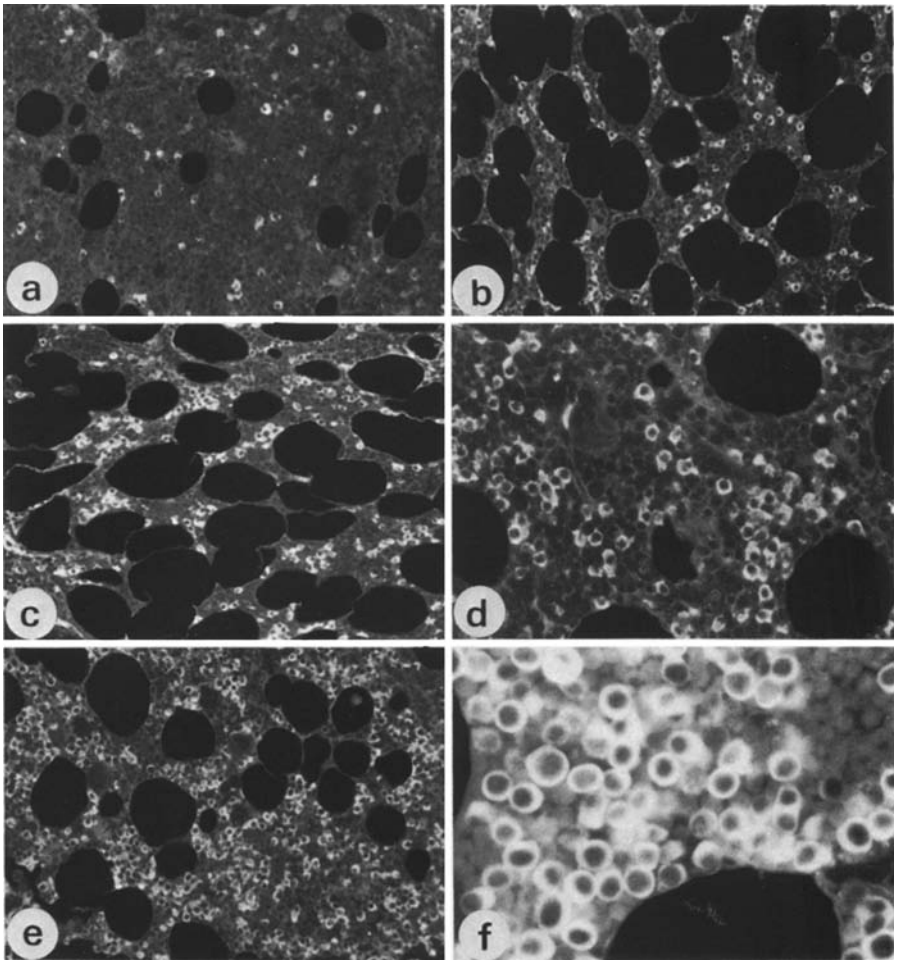

Fig. 5. Immunofluorescent photographs of immunoglobulin Ig-G containing cells in human bone marrow. **a** a 14-year old control showing no clustering (× 200). **b** 78-year old male control showing slight clustering (× 200). **c** Benign monoclonal gammopathy (Ig-G type) in 72-year old female patient showing scattered presence of clustered positive cells (× 200). **d** Higher magnification of the former case-(**c**) (× 400). **e** Multiple myeloma in a 72-year old female patient, showing extensive proliferation of positive cells (× 200). **f** Higher magnification of the former case-(**e**) showing numerous lymphoid positive cells with narrow cytoplasm (× 1000)

Table 2. Serum level of immunoglobulins and counts of immunoglobulin containing cells in bone marrow of benign monoclonal gammopathy (BMG) and multiple myeloma (MM) compared with age-matched control

	Number of cases	Serum Ig levels			Ig containing cells		
		Ig-G	Ig-A	Ig-M	G-cell	A-cell	M-cell
IgG-BMG	6	2675±381 (1.81±0.25) [43.2±8.9*]	138.7±46.2 (0.51±0.14)	74.83±16.4 (0.54±0.13)	68.2 ±9.68 (4.79±0.67)	3.33 ±1.00 (0.38±0.10)	1.17 ±0.41 (0.76±0.30)
IgG-MM	7	4436±231 (3.22±0.15) [19.6±3.2*]	47.14±13.7 (0.17±0.04)	37.28±7.13 (0.28±0.06)	269 ±36.2 (18.6±2.39)	0.71 ±0.31 (0.09±0.04)	0.28 ±0.18 (0.18±0.12)

Numbers without parentheses indicate absolute level of serum immunoglobulin or counts of immunoglobulin containing cells per unit field in the bone marrow.
Numbers in parentheses indicate relative ratio as compared with age-matched control. Asterisk (*) indicates the average ratio of serum level of M component divided by the number of Ig-G containing cells per unit field.
All numbers, mean ± S.E.M.

pattern of age-related change in the number of Ig-A containing cells in bone marrow was quite different from that of serum level of Ig-A, which showed only a slight, gradual increase with the advance of age. The pattern of distribution of both Ig-G and Ig-A containing cells showed a trend to clustering, the clusters increasing in number as well as in size with the advance in years (Figs. 3, 4). Thus, immunoglobulin containing cells were dispersed independently in the bone marrow of younger individuals (Fig. 5a), but compact clusters of 3 to 5 cells began to be observed after the 5th decade (Fig. 5a, b). Such a trend of cell accumulation was not usually observed in Ig-M containing cells.

Immunoglobulin containing cells in the bone marrow of benign monoclonal gammopathy (BMG) and multiple myeloma (MM)

In the bone marrow of BMG of Ig-G type (Fig. 5-c, -d), the number of Ig-G positive cells was 68.2±9.7 per unit field, which was significantly higher than control at any age (5–15). In contrast, the number of Ig-A and Ig-M positive cells was 3.3±1.0 and 1.2±0.4 respectively, apparently lower than that of adult controls.

In the bone marrow of MM of Ig-G type (Fig. 5-e, -f), the increase of Ig-G positive cells was more pronounced, showing 269.0±36.2 in average number, and the numbers of Ig-A and Ig-M positive cells were prominently suppressed, 0.7±0.3 and 0.3±0.2, respectively. The average serum level of Ig-G in these patients was 2,675±381 mg/dl in BMG and 4,436±231 mg/dl in MM. Thus, the average ratio of serum level of M-component divided by the number of Ig-G positive cells was 43.2±8.9 in BMG and 19.6±3.2 in

MM (Table 2), showing a significant difference between these ($p < 0.05$). Regarding the pattern of distribution, a trend to cluster formation was prominent in both BMG and MM. But the number of positive cells per cluster was apparently larger in MM (more than 50 cells) than in BMG (less than 20) (Fig. 5-d, -e). Lymphoid cells with narrow positive cytoplasm were frequently observed in MM (Fig. 5-f), but not in BMG.

Discussion

There have been many reports on age-related changes of human serum levels of immunoglobulin (Kalff 1970; Buckley and Dorsey 1970; Finger et al. 1973; Kishimoto et al. 1978). Most of these reports revealed that serum level of Ig-G and Ig-A increased in proportion to age without significant difference between sexes, but with marginal change in Ig-M. These findings were again confirmed in the present study.

Hijmans et al. (1971) examined the number of Ig-G, Ig-A and Ig-M producing cells in bone marrow smears by the immunofluorescent method and reported that the proportion of three types of immunoglobulin producing cells in the bone marrow was comparable with the rate of immunoglobulin production (Ig-G, 30 mg/kg/day; Ig-A, 27 mg/kg/day; Ig-M, 6 mg/kg/day) which were estimated by isotope labeling method (Waldman and Strober 1969).

In the present study, both Ig-G and Ig-A containing cells in human bone marrow increased in density with the advance of age. Such a phenomenon is partly consistent with the report that all classes of immunoglobulin producing cells (Ig-G, Ig-M, Ig-A) accumulate in the bone marrow of mice with increasing age (Benner and Haaijman 1980). Comparing the age-related pattern between the serum level of Ig-G and the density of Ig-G containing cells in bone marrow, the bone marrow appears to be one of the major sites of serum Ig-G production, as suggested by McMillan et al. (1972).

Another interesting finding is an increase of clustering of immunoglobulin containing cells with advancing age. Homogeneity of heavy as well as light chain in an individual clone may suggest the mono- or oligoclonal character of these clusters.

The presence of immunoglobulin containing cells in the bone marrow of adult mice and their deficiency in a germ free mouse (Benner et al. 1981) suggests that the immunoglobulins produced in the bone marrow are directed against exogenous antigens. In a young individual, antibody response caused by an antigenic stimulation can terminate after a certain interval due to the regulatory functions of the immune system, but in old individual the antibody response may not terminate promptly due to deficiency of the immunoregulatory function which declines with age (Makinodan and Kay, 1980). Radl (1979, 1981) presented a hypothesis that idiopathic paraproteinemia, the frequency of which increases with age, is a consequence of an age-related deficiency in the immune system. Thus the clustering of immunoglobulin containing cells in the marrow, which increases with age could be

considered to be an early change which may develop into idiopathic paraproteinemia.

Benign monoclonal gammopathy or idiopathic paraproteinemia is sometimes difficult to differentiate from multiple myeloma, although theoretically the latter is B cell malignancy and quite different from the former. There have been several reports indicating the difference between these two diseases. Lindstrom et al. (1973) showed a significant lowering of percentages of B cells having normal surface immunoglobulin in multiple myeloma as compared with benign monoclonal gammopathy. Using Feulgen staining of bone marrow smears to visualize nuclei and nucleoli, Turesson (1975) indicated that plasma cells in multiple myeloma had large nucleoli, large nuclei and an increased ratio of nucleolar/nuclear area. Durie et al. (1980) performed short term culture of bone marrow cells with the presence of H3-thymidine and reported that the labeling index in patients with benign monoclonal gammopathy was very low when compared with that in multiple myeloma. In spite of these suggestions mentioned above, Hickman and Avioli (1982) concluded that periodic examination of a patient with a monoclonal immunoglobulin is the only certain method of differential diagnosis.

In the present study, three points were suggested for differential diagnosis of BMG from MM. First, the ratio of serum level of M-component divided by the number of immunoglobulin containing cells per unit field was significantly higher in benign monoclonal gammopathy than in multiple myeloma and this index appeared to be useful for differential diagnosis. The low level of this index in patients with multiple myeloma may directly indicate low immunoglobulin in synthesis per myeloma cell, or could be ascribed to suppressor cells (Broder et al. 1975). Second, small round cells positive by immunofluorescent staining as described by Van Camp et al. (1981), were more prominent in multiple myeloma than in benign monoclonal gammopathy. Third, the number of cells in a cluster in multiple myeloma was generally over 50, but that in BMG less than 20, indicating that the size of cluster of immunoglobulin containing cells could be also useful in differential diagnosis. The limited cell counts in cell cluster of BMG may reflect the regulated growth of plasma cells, which is quite different from unlimited growth of myeloma cells.

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