Distribution of IgA₁ and IgA₂ subclasses in normal bone marrow trephines and in trephines infiltrated by IgA producing multiple myeloma

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SUMMARY A series of 20 bone marrow trephines biopsy and necropsy specimens were stained for IgA₁ and IgA₂ activity, together with total IgA, by means of an indirect immunoperoxidase technique using murine monoclonal antibodies applied to paraffin sections. The specimens showed normal histology and had been taken from patients not known to be suffering from haematological or related systemic disease. The IgA₁, IgA₂, and total IgA containing cells were counted and expressed as a percentage of all nucleate cells in the marrow cavities. Remarkably constant percentages of and ratios between these cell types were found. The same was true for a further 10 trephines taken from patients undergoing staging procedures for epithelial malignancies, where the marrow histology was normal. The pooled mean percentage of IgA₁ containing cells from both groups was 1·18% of all cells, that for IgA₂ containing cells 0·18%, and for total IgA positive cells 1·41%. In addition, 12 trephines containing known IgA producing myeloma were examined. Of these, 11 contained IgA₁, the remainder contained IgA₂ subclass.

The distribution of the two subclasses of immunoglobulin A (IgA) has previously been studied in cell suspensions from lymphoid and related tissues.^{1,2} The possibility that IgA₁ and IgA₂ could be shown in paraffin wax embedded tissue sections is of interest, as this is a less cumbersome technique than cell suspension analysis. As the distribution of these IgA subclasses may be important in, for example, immunological¹ or neoplastic disease we decided that a quantitative study of cells containing IgA₁ and IgA₂ in normal marrow trephine biopsies was appropriate. Paraffin wax embedded marrow trephines have previously been shown to be very well suited to immunohistochemical analysis.^{3–7} Furthermore, quantitative analysis of Ig classes has been carried out in this context.³

In addition to a study of IgA subclass distribution in normal marrow trephines, the histological investigation of multiple myeloma secreting IgA is also of interest, as, to the best of our knowledge, quantitative studies of IgA subclass distribution have not yet been performed *in situ* on bone marrow trephines, using immunoperoxidase methodology.

Material and methods

BONE MARROW SPECIMENS

Bone marrow specimens were removed 24–48 hours after death from 20 patients. Patients were selected on the basis of being previously healthy and having been killed suddenly in road traffic accidents. Fifteen of the patients were male; five female. The mean age was 40 years. Furthermore, 10 specimens were examined from 10 patients who were undergoing staging procedures for epithelial malignancy, where the marrow trephine was morphologically normal. Six of these patients were male; four female. Their mean age was 55.

Twelve trephines were also examined, which had been taken from patients (five men, seven women) with established IgA producing multiple myeloma. The mean age was 53 years.

A thin (5 mm thick) slice of bone marrow was removed from the anterior iliac crest from necropsy cases and fixed at room temperature in 5% glacial acetic acid in 10% formalin: this mixture simultaneously fixes and enhances immunostaining.³ For the staging and myeloma specimens, Jamshidi needle specimens were taken from the anterior iliac crest and similarly processed. Afterwards the specimens were

Fig 1 Normal bone marrow trephine. Six IgA₁ subclass containing cells are present (arrows). (Peroxidase reaction with monoclonal antibody to IgA₁.) × 200.

Fig 2 Normal bone marrow trephine. One IgA₂ subclass containing cells is present (arrow). (Peroxidase reaction with monoclonal antibody to IgA₂.) × 200.

routinely processed to paraffin wax. Sections were cut at a thickness of 2 μ m and submitted to the immunostaining procedure described below.

IMMUNOSTAINING

A standard indirect immunoperoxidase sequence was applied. Endogenous peroxidase activity was initially blocked by placing the slides in 0.3% hydrogen peroxide in methanol for 30 minutes. After a thorough wash in Tris buffer (pH 7.6) murine monoclonal antibodies to IgA₁, IgA₂, and total IgA were applied, at a titre of 1/50, at room temperature, for 40 minutes. (The antibodies were kindly supplied by Dr R Jefferis of the department of immunology, University of Birmingham). After further washes in Tris buffer (pH 7.6) the sections were overlaid with peroxidase conjugated rabbit antimouse IgM for 40 minutes at room temperature. A further wash in Tris buffer followed. Finally, the peroxidase was visualised by means of the 3,3'-diamino benzidine reaction. The sections were counterstained with Mayer's haemalum, dehydrated, cleared and mounted in synthetic medium. The usual controls, including adsorption studies, with appropriate IgA, IgA₁, and IgA₂ antigens, were performed.

COUNTING PROCEDURE

For each normal specimen, 1000 total cells were counted in the marrow spaces. For each of these thousand cells, those staining for IgA₁, IgA₂, or total IgA were enumerated and expressed as a percentage of all cells. A simple eyepiece graticule was used to avoid

recounting of cells, and all counting procedures were carried out on random fields using a 25 $\times$ objective lens. For the specimens of IgA myeloma, the sections were examined and the subclass of IgA being produced by the malignant cells was determined.

Results

NORMAL TREPHINES—CADAVERS

In all instances (as in the staging biopsies below), IgA₁, IgA₂, and total IgA containing cells were readily and clearly visualised. Their morphology was that of mature, Marschalkò type plasma cells, and they often occurred in small clusters. Virtually no "background" reaction was evident (figs 1 and 2). The staining with each antibody was "blocked" by its corresponding IgA subclass antigen but not by the other subclass antigen. Furthermore, staining of serial sections did not show IgA₁ and IgA₂ subclasses in the same cell.

The mean percentage of all cells that stained for IgA₁ was 1.19. The corresponding percentage for IgA₂ containing cells was 0.19. The percentage of all cells containing total IgA was 1.37. The cells containing IgA₁ formed a mean of 85.7% of all cells staining for total IgA, and those containing IgA₂ comprised 13.6% of all total IgA containing cells. (table 1) The interval between the time of death and the removal of the marrow specimens did not affect the results of immunostaining.

Table 1 Specimens studied and percentages of IgA₁, IgA₂ and total IgA containing cells in normal trephines (necropsy specimens)

Specimen (normal trephines)	Age (years)	Sex	Percentage of all cells containing IgA ₁	Percentage of all cells containing IgA ₂	Ratio of IgA ₂ positive: IgA ₁ positive cells	Percentage of all cells containing total IgA	Percentage of all cells containing IgA which are IgA ₂ positive	Percentage of all cells containing IgA which are IgA ₁ positive
1	16	M	1.11	0.17	15	1.30	13.1	85.4
2	18	M	1.33	0.19	14	1.48	12.8	89.9
3	18	F	1.21	0.17	14	1.39	12.2	87.1
4	22	M	1.10	0.28	25	1.36	20.6	80.9
5	24	M	1.08	0.22	20	1.34	16.4	80.6
6	27	M	1.30	0.09	7	1.39	6.5	93.5
7	27	M	1.21	0.16	13	1.35	11.9	89.6
8	30	F	1.19	0.21	18	1.41	14.9	84.4
9	31	M	1.18	0.11	9	1.28	8.6	92.2
10	33	M	1.05	0.31	30	1.37	22.6	76.6
11	36	M	1.09	0.18	17	1.29	13.9	84.5
12	42	M	1.13	0.21	19	1.34	15.7	71.9
13	47	M	1.21	0.29	24	1.52	19.1	79.6
14	47	M	1.27	0.16	13	1.40	11.4	90.7
15	51	F	1.33	0.15	11	1.46	10.3	91.1
16	57	F	1.31	0.11	9	1.42	7.7	92.3
17	61	M	1.19	0.13	11	1.34	9.7	88.8
18	72	M	1.21	0.21	17	1.40	15.0	86.4
19	73	F	1.04	0.28	27	1.35	20.7	77.0
20	75	M	1.20	0.12	10	1.30	9.2	92.3
Mean	40		1.19	0.19	16	1.37	13.6	85.7
SD	18.7		0.09	0.06	6.26	0.062	4.4	6.03
SEM	4.29		0.02	0.014	1.44	0.014	1.02	1.38

Distribution of IgA₁ and IgA₂ in normal and malignant bone marrow

Table 2 Specimens studied and percentages of IgA₁, IgA₂, and total IgA containing cells in normal trephines (staging biopsies)

Specimen	Age	Sex	Percentage of all cells containing IgA ₁	Percentage of all cells containing IgA ₂	Ratio of IgA ₂ positive: IgA ₁ positive cells	Percentage of all containing total IgA	Percentage of all cells containing IgA which are IgA ₂ positive	Percentage of all cells containing IgA which are IgA ₁ positive
1	44	M	1.10	0.16	15	1.41	11.3	78.0
2	44	F	1.12	0.18	16	1.37	13.1	81.8
3	48	F	1.14	0.19	17	1.52	12.5	75.0
4	49	M	1.21	0.16	13	1.46	11.0	82.9
5	49	M	1.10	0.22	20	1.39	15.8	79.1
6	50	F	1.28	0.24	19	1.40	17.1	91.4
7	51	M	1.15	0.18	16	1.30	9.2	88.5
8	58	M	1.20	0.12	10	1.71	7.1	70.2
9	62	F	1.19	0.16	13	1.41	11.3	84.4
10	63	M	1.22	0.14	7	1.38	10.1	88.4
Mean	52		1.17	0.17	17	1.44	11.85	81.9
SD	6.5		0.05	0.04	1.21	0.11	2.81	6.25
SEM	2.03		0.016	0.013	0.38	0.034	0.88	1.95

NORMAL TREPHINES—STAGING BIOPSIES

IgA₁ containing cells formed a mean of 1.17% of all cells and this mean percentage for IgA₂ containing cells was 0.17%. The percentage of all cells that stained for total IgA was 1.44. IgA₁ containing cells formed a mean of 81.9% of all total IgA containing cells, and the corresponding figure for IgA₂ was 11.85% (table 2). These data corresponded closely with those derived from necropsy tissue.

IGA MYELOMAS

In all specimens the marrow cavities were diffusely

infiltrated by sheets of myeloma cells. These stained strongly for total IgA and IgA₁ or IgA₂ subclasses in all cases. Staining for both subclasses was not seen in any specimens (figs 3 and 4). Of the 12 specimens, 11 stained for IgA₁, the remaining case reacting for IgA₂.

Discussion

In man IgA exists as two antigenically different subclasses, IgA₁ and IgA₂. IgA₁ has been shown to account for about 90% of total serum IgA but it may

Fig 3 Bone marrow infiltrated by IgA myeloma. Infiltrating cells contain IgA₁ (Peroxidase reaction with monoclonal antibody to IgA₁). × 400.

Fig 4 Bone marrow infiltrated by IgA myeloma from same specimen as in fig 3. Cells do not contain IgA₂. (Peroxidase reaction with monoclonal antibody to IgA₂.) $\times 400$.

be less predominant in other fluids, such as colostrum, where IgA₁ constitutes 70% or less of total IgA.⁸ Using immunofluorescence on frozen sections, a previous study¹ showed that in mucosal and glandular tissues the percentages of IgA₁ and IgA₂ containing plasma cells, in relation to total IgA containing cells, more closely approximated to those in colostrum rather than those in serum. In peripheral lymph nodes, however, the proportions were closer to those observed in serum and in marrow cells in suspension.⁹ Even in normal subjects, therefore, the ratio of IgA₁:IgA₂ bearing cells is not constant for all tissues and fluids. IgA₂ possesses greater resistance than IgA₁ to cleavage by the IgA protease of streptococci, which may, teleologically, suggest that its biological role in secretions such as colostrum is highly important.¹⁰

Monoclonal antibodies to both IgA subclasses have been used in a previous study to examine the differentiation of human peripheral blood B cells expressing IgA.¹¹ It was found that in the human neonate there were equal proportions of IgA₁ and IgA₂ bearing B cells. These cells also expressed surface IgM, but with increasing age the IgA containing cells lost IgM and showed a great increase in the proportion of IgA₁ containing cells (98% in infants falling to 80% in adults) in relation to those expressing IgA₂.

Our study has shown that the three monoclonal

antibodies used can readily be applied to paraffin embedded tissue sections. They apparently do not cross react, as staining for IgA₁ and IgA₂ was not observed in the same cell in serial sections cut at 2 μ m thickness. Furthermore, the numbers of IgA₁ and IgA₂ containing plasma cells were remarkably constant in the two groups of morphologically normal trephines. The numbers of total IgA containing cells, expressed as mean percentages of all cells in the cadaveric and biopsy marrows—namely, 1.37 and 1.44, respectively—agreed with the results of a previous study of IgA (and other Ig class) containing cells in normal trephines.³ In normal trephines IgA containing cells accounted for 1.28% of all cells when “stained” with a polyclonal antiserum directed against α heavy chain.

The constancy of the current results could suggest that the ratio of IgA₁:IgA₂ bearing cells is in some way regulated in the normal marrow and is closely allied to the ratio seen in peripheral lymphoid tissues and serum. The same is true of the absolute numbers of these cells when compared with those of a previous study of cell suspensions of bone marrow and other lymphoid organs using immunofluorescence.²

The monoclonal antibodies used in this study have been highly characterised as part of a previous study of immunogenic and antigenic epitopes, including a range of antibodies specific for human IgA, IgA₁, and IgA₂ subclasses and an nA2m(2) isoallotypic epit-

ope.¹² They have also proved to be of great value in the study of IgA subclass distribution in normal and diseased lungs,¹³ where IgA containing plasma cells (especially those with IgA₁) were shown to be greatly increased in chronic bronchitis.

The occurrence of IgA synthesis by multiple myeloma is well recognised and is seen in about 20% of all specimens,¹⁴ being second in frequency to IgG secreting myelomas. The two subclasses of IgA were originally discovered in IgA myeloma¹⁵ and, indeed, antibodies to IgA₁ and IgA₂ have generally been prepared against myeloma protein.^{11,12} We observed that most of our series of IgA myelomas were expressing IgA₁ rather than IgA₂. This may reflect a simple probabilistic phenomenon, where IgA₁ containing cells, being more common than IgA₂ containing cells in healthy subjects, are also more likely to become malignant. Certainly, our results reflect those obtained serologically in patients with IgA myeloma.

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References

- 1 Andre C, Andre F, Fargier MC. Distribution of IgA₁ and IgA₂ plasma cells in various normal human tissues and in the jejunum of plasma IgA-deficient patients. *Clin Exp Immunol* 1978;33:327-31.
- 2 Crago SS, Kuttah WH, Prince SJ, Radl J, Haaijman JJ, Mestecky J. Distribution of IgA₁ and IgA₂ subclasses in human tissue: correlation with the presence of J-chain. In: McGhee JR, Mestecky J, eds. *The secretory immune system* 1983:803-5.
- 3 Crocker J, Curran RC. Quantitative study of the immunoglobulin-containing cells in trephine samples of bone marrow. *J Clin Pathol* 1981;34:1080-2.
- 4 Crocker J, Jenkins R, Burnett D. Immunohistochemical demonstration of leucocyte elastase in human tissues. *J Clin Pathol* 1984;37:1114-8.
- 5 Crocker J, Jenkins R, Burnett D. Immunohistochemical localisation of cathepsin G in human tissues. *Am J Surg Pathol* 1985;9:338-43.
- 6 Crocker J, Smith PJ. Value of factor VIII-related antigen as a means of demonstrating extramedullary megakaryopoiesis. *J Clin Pathol* 1984;37:834-5.
- 7 Sangster G, Crocker J, Nar P, Leyland MJ. Benign and malignant (B cell) focal lymphoid aggregates in bone marrow trephines shown by immunogold-silver technique. *J Clin Pathol* 1986;39:453-7.
- 8 Grey HM, Abel CA, Yount WJ, Kunkel HG. A subclass of human A-globulins (A2) which lacks the disulfide bonds linking heavy and light chains. *J Exp Med* 1968;128:1223-6.
- 9 Skvaril F, Morell A. Distribution of IgA subclasses in sera and bone marrow plasma cells of 21 normal individuals. In: Mestecky J, Lawton AR, eds. *The immunoglobulin A system. Advances in Experimental Medicine and Biology*. New York: Plenum Press, 1974:433.
- 10 Plaut AG, Wistar R, Capra JD. Differential susceptibility of human IgA immunoglobulins to streptococcal IgA protease. *J Clin Invest* 1974;54:1295-3000.
- 11 Conley ME, Kearney JF, Lawton AR, Cooper MD. Differentiation of human B cells expressing the IgA subclasses as demonstrated by monoclonal hybridoma antibodies. *J Immunol* 1980;125:2311-6.
- 12 Farris MA, Hardie D, Lange G de, Jefferis R. Immunogenic and antigenic epitopes of immunoglobulins X: monoclonal antibodies specific for human IgA, the IgA₁ and IgA₂ subclasses and an nA2m(2) iso-allotypic epitope. *Vox Sang* 1985;48:116-21.
- 13 Burnett D, Crocker J, Jenkins R, Stockley RA. IgA₁ and IgA₂ plasma cells in the bronchial walls of subjects with and without chronic bronchitis. *Am Rev Respir Dis* 1985;131:22.
- 14 Pruzanski W, Ogryzlo MA. Abnormal proteins in malignant diseases. *Adv Clin Chem* 1970;13:355.
- 15 Feinstein D, Franklin EC. Two antigenically distinguishable subclasses of human γ A myeloma proteins differing in their heavy chains. *Nature* 1966;212:1496-8.

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