Cytogenetic Studies in a Family with Waldenström's Macroglobulinaemia

MICHAEL W. ELVES* and ALAN K. BROWN

From the Department of Medical Genetics, Manchester University; and Ancoats Hospital, Manchester

Waldenström's macroglobulinaemia is a disease characterized by an increase in the serum of gammaglobulins of high molecular weight (macroglobulin). The chief feature of the bone-marrow of these patients is an infiltration of numbers of small lymphocytes. The clinical and pathological features of primary macroglobulinaemia have been described by several authors since the syndrome was first defined by Waldenström (Waldenström, 1944, 1948; Imhof, Baars, and Verloop, 1959; Mackay, 1959; Martin, 1960; Kok, Whitmore, and Ainsworth, 1963).

A serological and cytogenetic study has been carried out on the relatives of a patient with Waldenström's macroglobulinaemia. The detailed clinical and serological aspects of the family have been presented elsewhere (Brown *et al.*, 1967), and therefore only brief details of these aspects of the family will be given here. The purpose of this article is to describe in full and to discuss the cytogenetic abnormalities found in this family, particularly with regard to the incidence of pseudodiploidy.

Material and Methods

Paper electrophoresis of the serum proteins was carried out by the method of Tiselius (1939) and immunoelectrophoresis by the method of Scheidegger (1955), using horse anti-human serum (Pasteur Institute). Antiglobulin neutralization tests (Kekwick *et al.*, 1961) were carried out, using a reagent containing anti- γ_1 and anti- γ_2 , and red cells sensitized with anti-D to detect γ_2 -globulin, and cells coated with anti-Le^a in the absence of complement (Stratton, Gunson, and Rawlinson, 1962). Sia test was carried out by the method of Martin (1960). Blood samples were taken from available members of the family and chromosomes were obtained from cultured lymphocytes, according to the method of Hungerford *et al.* (1959). All cells containing the abnormal marker chromosome were examined directly with the microscope and then from

photomicrographs. Cells with a modal number of chromosomes were examined in the same way, and in these cases particular attention was given to the No. 2 chromosome pair, for reasons which will become apparent.

Case Reports

This family will be fully described elsewhere; only brief details are given here. The relationship of the members of the family to the proband (II. 1) is shown in Fig. 1. All were studied, except II. 3 who died of congestive heart failure early in the investigation.

FIG. 1. Pedigree of a family showing protein abnormalities and chromosomal abnormalities.

II. 1, aged 62 years. This female patient showed the characteristic features of Waldenström's macroglobulinaemia. The onset of the illness was insidious, with weight loss, lassitude, anaemia, spontaneous bleeding, and cardiac failure. The diagnosis was suggested by a greatly increased erythrocyte sedimentation rate and by the presence of a dense band in the γ -region on paper electrophoretic examination of the serum. The Sia water test was positive. Immunological studies and ultracentrifugal examination confirmed the diagnosis.

The patient died three years after the initial examination and necropsy showed the typical, diffuse, pleomorphic infiltrate in the marrow and lymph nodes.

Six relatives were screened by clinical examination, routine laboratory tests, and serological studies, and one relative (II. 2) showed a serum protein abnormality. A second relative (III. 5) showed a slight increase in γ -globulin. Details are shown in Table I.

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* Present address: Charles Salt Research Centre, The Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire.

TABLE I
PATHOLOGICAL DATA IN THE RELATIVES

Case	Hb (g./ 100 ml.)	WBC per c.mm.	ESR mm./hr. (West'g'n)	Latex Agg'n Tests		Blood Group	Total Serum Proteins (g./ 100 ml.)	Serum Alb. (g./ 100 ml.)	Serum Glob. (g./ 100 ml.)	Sia Test	Paper Electro- phoresis	W.R.	Immuno- electro- phoresis	Antiglob. Neutral'n Test
				R.A.	L.E.									
II. 2	12.6	4400	38	-ve	-ve	A Rh +ve	7.7	3.7	4.0	-ve	Inc'd band γ -region	-ve	Prominent γ_1 -glob. band	γ_1 -glob. inc'd
III. 3	13.4	3200	3	-ve	-ve	A Rh +ve	6.9	3.9	3.0	-ve	Normal	-ve	Normal	Normal
III. 2	13.0	4200	2	-ve	-ve	A Rh +ve	6.8	4.0	2.8	-ve	Normal	-ve	Normal	Normal
III. 1	13.4	6800	1	-ve	-ve	A Rh +ve	6.5	3.8	2.7	-ve	Normal	-ve	Normal	Normal
III. 4	12.7	5400	2	-ve	-ve	O Rh +ve	6.2	3.6	2.6	-ve	Normal	-ve	Normal	Normal
III. 5	11.7	6200	3	-ve	-ve	A Rh +ve	6.8	3.6	3.2	-ve	Slightly inc'd band γ -region	-ve	Normal	Normal

II. 2, aged 58 years. This female patient had a history of encephalitis two years before the present study. At that time serum protein estimation was normal and the erythrocyte sedimentation rate was 28 mm. in one hour (Westergren). Two years later the total serum proteins were 7.7 g./100 ml. with a serum globulin level of 4 g./100 ml. The erythrocyte sedimentation rate was 38 mm. in one hour (Westergren), and paper electrophoresis of the serum showed a dense band in the γ -region. Immuno-electrophoretic and antiglobulin neutralization tests (kindly performed by Dr. H. H. Gunson of the Blood Transfusion Service, Manchester) showed an increase in γ_1 -globulin.

No clinical or serological abnormalities were found in the remaining five relatives who were investigated.

Cytogenetic Data

II. 1 had 2 cells with 47 chromosomes, the additional autosome being larger than any others in the karyotype and typical of that found in some other cases of macroglobulinaemia. In one cell this abnormal chromosome was metacentric and in the second it was submetacentric (Fig. 2). Two pseudodiploid cells were also found, which contained a large submetacentric chromosome. In one of these cells a No. 21-22 chromosome was missing, while in the second a No. 2 chromosome was absent. In this latter cell it was thought that the macroglobulinaemia chromosome (M chromosome) was the missing

No. 2, but on comparing them, the M chromosome was 26% longer than the No. 2. The incidence of the abnormality in this patient was therefore 8%.

In **II. 2** no cells containing a typical macroglobulinaemia chromosome complement were seen (i.e. 47 chromosomes, the supernumerary autosome being either a large metacentric or submetacentric chromosome). There were however a number of pseudodiploid cells which contained a large submetacentric chromosome. In two of these a No. 2 chromosome was absent and the M chromosome was 22% larger than the No. 2 present; they were therefore not considered to be homologous, as they were also morphologically dissimilar. Another cell had a submetacentric M chromosome, but one of the small autosomes of group 19-20 was absent. In a fourth cell a similar M chromosome was present, but a small member of group 6-12 (?12) was absent. 16% of the cells, therefore, carried an M chromosome.

In **III. 3**, no abnormal cells were seen.

In **III. 1**, no typical 'macroglobulinaemia' karyotype was found. One pseudodiploid cell had a submetacentric M chromosome, but a No. 2 autosome was absent. Again the M chromosome was not considered to be the missing No. 2, as it was 28% longer. Two cells had a similar M chromosome, but a small member of group 6-12 (? 12) was missing. 3% of the cells were, therefore, abnormal.

In **III. 2**, two cells with 47 chromosomes containing an additional large chromosome were seen. In one cell

FIG. 2. Chromosomes of groups 1-5 from a cell containing a submetacentric M chromosome.

FIG. 3. Karyotype of cell from III. 5 (G.W.J.), showing an abnormal M chromosome and a small chromosome missing from group 6-12.

a submetacentric M chromosome was found, and in the other a smaller submetacentric chromosome, which was the same size and shape as the No. 3 pair, was present. Two cells were pseudodiploid, a submetacentric M chromosome replacing a No. 2, and in these cells the M chromosomes were 18% and 16% longer than the normal No. 2. The incidence of abnormal cells was therefore 5.3%.

In III. 5, one typical macroglobulinaemia cell was seen which contained an extra submetacentric chromosome. In addition, 3 pseudodiploid cells were found. In one a submetacentric M chromosome replaced a No. 2 and was 23% longer than the No. 2 chromosome present. In a second the submetacentric M chromosome was present and a small member of group 6-12 (? 12) was absent (Fig. 3). In the third, a similar M chromosome was present, but a member of group 17-18 was present. The incidence of abnormal cells was therefore 8%.

In III. 4, no abnormal cells were seen. These results are summarized in Tables II and III.

TABLE II
RESULTS OF CHROMOSOME COUNTS IN FAMILY

Case	Distribution (No. of mitoses)						
	44	44	45	46	47	48	48
II. 1	3	3	4	38	2	0	0
II. 2	1	2	1	21	0	0	0
III. 1	2	0	2	21	0	0	0
III. 2	1	1	1	20	2	0	0
III. 3	1	2	2	20	0	1	0
III. 4	1	1	2	20	0	1	0
III. 5	2	0	3	19	1	0	0

Discussion

Apart from the propositus only one other member of the family has an abnormal serum globulin pattern, in which the γ_1 fraction was raised. III. 5 had a slight increase in gammaglobulin on electrophoresis, but this has not been confirmed by the antiglobulin neutralization test. We cannot therefore at present regard him as abnormal.

There are two studies of familial serum protein abnormalities associated with macroglobulinaemia (Massari, Fine, and Metais, 1962; Seligmann and Badin, 1962). No cytogenetic studies have been made in such families as far as we are aware. Chromosome abnormalities have now been found in a number of cases of Waldenström's macroglobulinaemia (Bottura, Ferrari, and Veiga, 1961;

TABLE III
INCIDENCE OF ABNORMAL CELLS IN FAMILY

Case	No. of Cells Examined*	Abnormal Cells		Total Abnormal Cells	% Abnormal Cells
		47M	46M		
II. 1	50	2	2	4	8
II. 2	25	0	4	4	16
III. 1	100	0	3	3	3
III. 2	75	2	2	4	5.3
III. 3	50	0	0	0	0
III. 4	100	0	0	0	0
III. 5	50	1	3	4	8

* Includes cells in which complete counts were not possible, and which are not included in Table II, but in which all chromosomes of groups 1-5 (inc. M) could be seen.

German, Biro, and Bearn, 1961; Benirschke, Brownhill, and Ebaugh, 1962; Elves and Israëls, 1963). There is, however, no specific abnormality. The abnormal chromosome which is present in only a small proportion of dividing lymphocytes may be either a large metacentric, or a submetacentric chromosome. In one case only is the additional large chromosome almost acrocentric (Bottura *et al.*, 1961). Similar abnormalities may be found occasionally in subjects with mild degrees of hypergammaglobulinaemia without any evidence of macroglobulinaemia (Elves and Israëls, 1963).

The abnormal chromosome may therefore exist without any abnormal protein fraction being produced. This family is another example of this phenomenon. It is also the first in which the chromosomal abnormality has been shown to be familial. The abnormal chromosome is present in 5 out of the 7 subjects studied, and in all but one cell it is the large submetacentric variety (Type II). One metacentric chromosome (Type I) has been found in a cell from the *propositus*. Only in the *propositus* is there any evidence of excess macroglobulin production.

Of interest in this family is the incidence of pseudodiploidy. In a number of cells the abnormal chromosome is present, but the chromosome number remains modal (i.e. 46) due to the loss of one of the normal autosomes. It is questionable how much attention should be paid to these cells, particularly when the missing autosome is one of the smaller members of the karyotype. The absence of other similar cells, either from the same subject or from others, suggests that these cells may have suffered loss of a chromosome during the preparation of the chromosomes for study. Thus, it is perhaps more desirable to consider these cells as 'normal' macroglobulinaemia cells and not additional abnormal stem cell lines. Some pseudodiploid cell lines do appear consistently, however, both in different cells of the same subject and also in cells of different subjects. One of these abnormal cell lines possesses the Type II chromosome, but is deficient in a normal No. 2 autosome. The M chromosome was considered to be the second No. 2 autosome until it was found that it was consistently longer, the difference in size ranging from 16–28% of the mean length of the pair. As controls, cells from 10 normal unrelated subjects were examined and the percentage difference in length of the No. 2 homologues was estimated. The range of difference found was 0–11%, with a mean of 4%. This type of cell has been found in all of the abnormal subjects, including the *propositus* (Table II). The

second abnormal pseudodiploid cell type is deficient in a smaller member of group 6–12. This cell type is present in 4 subjects. We are reluctant to dismiss these cells as artefacts. Furthermore, Houston, Ritzmann, and Levin (1967), studying a series of patients with macroglobulinaemia, have found a high incidence of structural or numerical abnormalities in one of the smaller pairs of chromosomes in group 6–12. They also suggest that the No. 12 pair was involved in these abnormal cells.

As the abnormal chromosome in these blood cultures is confined to only a small proportion of cells of the lymphoid series, it is clear that the abnormal stem cell line originates during the later period of embryonic growth or in postnatal development. It is as yet uncertain whether or not it is only the lymphoid cell types that carry this abnormality, or whether there is a general mosaicism throughout all cells of the body. So far no examination has been made of the karyotype of skin cells in such patients, and we were unable to carry out this investigation in this family. In addition, it was not possible to obtain bone-marrow specimens from the relatives of the *propositus*. There is some evidence, however, that abnormal stem cells may be present in the bone-marrow. Bottura *et al.* (1961) reported the presence of a large chromosome in some of the cells obtained from a patient with this disease by sternal puncture, and others have also found abnormal cells in the marrow of patients with macroglobulinaemia (Heni and Siebner, 1963; Houston *et al.*, 1967).

The bone-marrow in this condition, however, usually contains increased numbers of small lymphoid cells, and it may be these cells or their precursors that carry the abnormal chromosome rather than cells of the erythrocyte or granulocyte series. Of interest in this context is the patient described by Houston *et al.* (1967) who, in addition to macroglobulinaemia, also had chronic myeloid leukaemia. Cells containing the abnormal chromosome were found in both blood cultures and direct bone-marrow samples from this patient. The authors were not able to identify with certainty the cell type represented by the abnormal mitotic figures, but the majority of cells in the marrow were of the myeloid series. Further, Ph¹ containing cells, which are thought at present to be non-lymphocytic, were also seen and contained the abnormal 'macroglobulinaemia' chromosome. Thus, it appears that the abnormal cell type is not necessarily confined to cells of the lymphoid series. It may be suggested that the pathological serum protein pattern in these patients is due to the existence in the lymphoid population of a 'clone' of cells which contain excess

genetic material controlling the synthesis of gamma-globulin (carried by the abnormal chromosome). Its presence in three members of this family in which no protein abnormality was detectable indicates that gammaglobulin overproduction is not an inevitable consequence of the presence of this chromosome. It can be postulated that the abnormal cell line may lie dormant until a suitable stimulus is encountered which normally induces gammaglobulin production, and, concomitant with this, abnormal gammaglobulin would also be formed. The nature of such a stimulus is not clear, but as there is evidence that the lymphocyte is concerned in immunological processes, it may be antigenic in nature. The encephalitis suffered by II.2 may have provided such a stimulus. At the time of her illness, this patient had normal gamma-globulin levels which are now raised.

An alternative hypothesis is that the abnormal cell line is suppressed by a homeostatic mechanism. If there was a breakdown of this immuno-suppressive mechanism, the abnormal cells would be able to proliferate. Phytohaemagglutinin, if it acted in a manner suggested by Coulson and Chalmers (1964), would short-circuit the recognition mechanism and would stimulate all immunologically competent cells to transform and divide. Thus, the blood culture technique provides an artificial system in which cell lines not normally active may be revealed.

Summary

Chromosome abnormalities were demonstrated in 4 out of 6 relatives of a patient with Waldenström's macroglobulinaemia. Apart from the propositus, only one other member of the family had a significantly abnormal serum protein abnormality in which the γ_1 fraction was increased. The abnormal cells contained a large submetacentric chromosome, except for one large metacentric chromosome which was found in a cell from the propositus.

Encephalitis was followed by a gammaglobulin abnormality in one of the relatives. An antigenic stimulus may initiate gammaglobulin overproduc-

tion or, alternatively, proliferation of abnormal cells may result from a disturbance of the normal immuno-suppressive mechanism.

REFERENCES

- Benirschke, K., Brownhill, L., and Ebaugh, F. G. (1962). Chromosomal abnormalities in Waldenström's macroglobulinaemia. *Lancet*, **1**, 594.
- Bottura, C., Ferrari, I., and Veiga, A. A. (1961). Chromosome abnormalities in Waldenström's macroglobulinaemia. *ibid.*, **1**, 1170.
- Brown, A. K., Elves, M. W., Gunsen, H., and Pell-Iderton, R. (1967). Familial macroglobulinaemia. *Acta haemat. (Basel)*, **38**, 184.
- Coulson, A. S., and Chalmers, D. G. (1964). Separation of viable lymphocytes from human blood. *Lancet*, **1**, 468.
- Elves, M. W., and Israëls, M. C. G. (1963). Chromosomes and serum proteins: a linked abnormality. *Brit. med. J.*, **2**, 1024.
- German, J. L., Biro, C. E., and Bearn, A. G. (1961). Chromosomal abnormalities in Waldenström's macroglobulinaemia. *Lancet*, **2**, 48.
- Heni, F., and Siebner, H. (1963). Chromosomenveränderungen bei der Makroglobulinämie Waldenström. *Dtsch. med. Wschr.*, **88**, 1781.
- Houston, E. W., Ritzmann, S. E., and Levin, W. C. (1967). Chromosomal aberrations common to three types of monoclonal gammopathies. *Blood*, **29**, 214.
- Hungerford, D. A., Donnelly, A. J., Nowell, P. C., and Beck, S. (1959). The chromosome constitution of a human phenotypic intersex. *Amer. J. hum. Genet.*, **11**, 215.
- Imhof, J. W., Baars, H., and Verloop, M. C. (1959). Clinical and haematological aspects of macroglobulinaemia Waldenström. *Acta med. scand.*, **163**, 349.
- Kekwick, R. A., Vallet, L., Cutbush, M., Mollison, P. L., Thomas, A. R., Gell, P. G. H., and Soothill, J. F. (1961). Estimation of gamma-globulin in the serum of patients with hypogammaglobulinaemia. *J. clin. Path.*, **14**, 470.
- Kok, D'A., Whitmore, D. N., and Ainsworth, R. W. (1963). Four cases of Waldenström's macroglobulinaemia. *ibid.*, **16**, 351.
- Mackay, I. R. (1959). Macroglobulins and macroglobulinaemia. *Aust. Ann. Med.*, **8**, 158.
- Martin, N. H. (1960). Macroglobulinaemia: a clinical and pathological study. *Quart. J. Med.*, **29**, 179.
- Massari, R., Fine, J. M., and Metais, R. (1962). Waldenström's macroglobulinaemia observed in two brothers. *Nature (Lond.)*, **196**, 176.
- Scheidegger, J. J. (1955). Une micro-méthode de l'immunoélectrophorèse. *Int. Arch. Allergy*, **7**, 103.
- Seligmann, M., and Badin, J. (1962). β 2-Macroglobulinémie familiale. *Rev. franç. Étud. clin. biol.*, **7**, 1107.
- Stratton, F., Gunson, H. H., and Rawlinson, V. I. (1962). The preparation and uses of antiglobulin reagents with reference to complement-fixing blood group antibodies. *Transfusion (Philad.)*, **2**, 135.
- Tiselius, A. (1939). *Harvey Lect.*, **35**, 57.
- Waldenström, J. (1944). Incipient myelomatosis or 'essential' hyperglobulinemia with fibrinogenopenia—a new syndrome. *Acta med. scand.*, **117**, 216.
- (1948). Zwei interessante Syndrome mit Hyperglobulinämie. (Purpura hyperglobulinaemica und Makroglobulinämie). *Schweiz. med. Wschr.*, **78**, 927.