Compared with the population in Great Britain, they showed an increased incidence of asthma and congenital abnormalities, but a striking absence of atherosclerosis. In other respects—for example, complaints of dyspepsia, blood-pressure levels—they showed the same trends as the population of Britain.

Among the congenital abnormalities the incidence of retinitis pigmentosa is most serious.

The significance of these findings should be borne in mind if the Tristanians continue as an inbred and isolated community.

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CHROMOSOMES AND SERUM PROTEINS: A LINKED ABNORMALITY

BY

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In recent years chromosome abnormalities have been reported in direct marrow preparations (Bottura *et al.*, 1961) and peripheral blood cultures (German *et al.*, 1961; Benirschke *et al.*, 1962) from patients with macroglobulinaemia. In the case reported by Bottura *et al.* (1961) the abnormal cell-line contained, in addition to the normal diploid complement, an abnormal large and almost acrocentric chromosome. In the other two cases the abnormal cell-line, with 47 chromosomes, comprised about 4% of the mitotic population of the cultures and the additional chromosome was a large metacentric or a submetacentric chromosome.

The relationship between the genetic abnormality and the biochemical defect in this disease has, up until now, not been clear. German *et al.* (1961) suggested that the biochemical abnormality may have induced the genetic damage; Patau (1961) did not agree with this view. We report one case of proved macroglobulinaemia in which a chromosomal abnormality was found and two further cases in which the incidental finding of chromosomal abnormality led to the discovery of dysproteinaemias. We believe that these three cases throw new light upon the relationship between these genetic abnormalities and the protein defect.

Case Reports

Case 1: macroglobulinaemia.—A man aged 66 presented with dyspnoea and cor pulmonale. On examination there was slight enlargement of both liver and spleen; there was no lymphadenopathy. The fundi oculi showed very full and tortuous veins but no haemorrhages. Blood count revealed a slight anaemia (Hb 8.2 g./100 ml.) but normal leucocyte and platelet counts. Sternal marrow biopsy revealed a moderately cellular marrow with all normal cell types represented, but in addition there were increased numbers of plasma cells, reticulum cells, and small lymphoid cells similar to those reported in cases of macroglobulinaemia. Serum globulin was 7.9 g./100 ml., and on electrophoresis there was an intense narrow band moving as a γ -globulin. The plasma solidified at 30° C. and the cryoglobulin test was strongly positive. Immunoelectrophoresis

and antiglobulin neutralization studies confirmed the identity of the abnormal protein as a γ -globulin. Ultracentrifuge studies were not successful owing to insolubility of the protein, but it was probably in the 19 Svedberg unit class. Treatment was instituted with chlorambucil (10 mg./day), and after five and a half months serum globulin concentration had fallen to 5.5 g./100 ml., and after eight months to 4.7 g./100 ml. Marrow biopsy at this time failed to reveal any excess of plasma cells or lymphoid cells.

Case 2.—A 24-year-old woman presented with increasing tiredness and pallor. She had given birth 10 months previously to a premature mongol infant who died after six months, and it was for this reason that the cytogenetic studies which revealed an abnormal chromosome were carried out. Nothing abnormal was found on either clinical or haematological examination. Owing to the presence of the abnormal chromosome serum protein studies were undertaken and a slight protein abnormality was found when serum was subjected to the antiglobulin neutralization test, which indicated a small increase in γ -globulin. The total amount of globulin present was, however, within normal limits (2.6 g./100 ml.). No macroglobulinaemia was found.

Case 3.—A 19-year-old woman had had recurrent purpura since the age of 17. Clinical and haematological examination at the time of study revealed no abnormality. Serum protein analysis at this time showed an increase in the amount of globulin (5.2 g./100 ml.), which was found by electrophoresis and the antiglobulin neutralization test to be due to an increase in γ -globulin. No macroglobulinaemia was detected. Because of the dysproteinaemia cytogenetic studies were undertaken.

Cytogenetic Studies

Cytogenetic studies were carried out on all three patients, using the peripheral blood-culture technique (Hungerford *et al.*, 1959). In Case 1 serial studies were made before treatment and after five and a half and eight months. A normal modal number of 46 was found in each case with no excessive degree of aneuploidy, although the percentage of aneuploid cells in Case 1 did increase during the course of treatment. These results are shown in the Table.

Two types of abnormal chromosomes were found in addition to the normal karyotype in cells with 47 chromosomes in these three patients. Both were larger than any other in the normal diploid set, but one was metacentric

Serum Globulin Levels and Cytogenetic Data

Case No.	Serum Globulin (g./100 ml.)	Chromosome Count Distribution (%)					No. of Cells Examined	Abnormal Cells		
		<45	45	46	47	<47		No.	Type	%
1	7.9*	0	8	84	8	0	50	2	I	4
	5.5†	20	8	68	4	0	70	1	I	1.4
	4.7‡	24	8	64	4	0	40	0		0
2	2.6	8	10	76	6	0	50	2	II	4
3	5.2	6	4	74	14	2	50	3	I	6
								4	II	8

* Pretreatment. † 5½ months' treatment. ‡ 8 months' treatment.

FIG. 1.—Abnormal karyotype in Case 1, showing additional large chromosome (type I).

FIG. 3.—Analysis of group 1-3 (+abnormal chromosome) in two cells from Case 3. Top cell contains a type II abnormal chromosome, the bottom a type I abnormality.

FIG. 2.—Abnormal karyotype in Case 2, showing additional large chromosome (type II).

in shape (type I) while the second was submetacentric (type II). In Case 1 the type I chromosome was seen in 4% of the mitoses in cultures made before treatment (see Fig. 1), and, as will be seen from the Table, the abnormal cell-line decreased as the serum globulin level was reduced. The type II abnormality was found in 4% of the mitoses in cultures from Case 2 (see Fig. 2). Case 3 was interesting because both types of abnormal chromosome were found, although never in the same cell: 6% of the mitotic figures contained a type I abnormality and 8% of type II (see Fig. 3).

Discussion

Abnormal chromosomes have now been found in at least four patients with macroglobulinaemia (Bottura *et al.*, 1961; German *et al.*, 1961; Bernirschke *et al.*, 1962; present case) in whom the abnormal cells constituted only 4-5% of the mitotic cells in lymphocyte cultures. As the mitotic cells formed less than 10% of the culture population at 72 hours the percentage of affected cells in the

entire lymphocyte population of the peripheral blood would be very small indeed, and such cells, even if they were morphologically abnormal, would easily escape detection.

In normal individuals macroglobulins constitute 2.5% of the serum proteins, and there is a close immunological relationship between this normal macroglobulin and that found in Waldenström's syndrome (Korngold and Leeuwen, 1957). In this disease there is no evidence of a decrease in production of normal globulins as there may be in myelomatosis, and, in fact, Martin (1960) has shown an increased production of both normal protein and macroglobulin in two patients. One feature of the disease is the finding, in marrow, lymph nodes, and other organs, of cells resembling small lymphocytes but with some morphological abnormalities: such cells were found in Case 1. These may be the cells responsible for the production of the abnormal protein and may also be carriers of the abnormal chromosomes.

Cases 1-3, when considered with others in the literature, lead to some speculation concerning the relationship between the genetic defect and the protein abnormality. Two alternatives now may exist: (1) that the abnormal chromosome and production of excess macroglobulin

are unrelated and the finding of both together in at least three individuals was fortuitous; and (2) that the abnormal chromosome confers upon the cell the ability to produce abnormal γ -globulin under certain conditions.

The first argument may be quickly disproved by the clear demonstration in Case 1 of the simultaneous decrease in both abnormal protein and cells with abnormal chromosomes during therapy. The two "normal patients" (Cases 2 and 3) who possess abnormal cell lines indicate that if the second argument is valid the genetic defect precedes the biochemical lesion perhaps by some years. It is also of interest to note that of these two patients the one with a "double dose" of genetic abnormality has a more pronounced dysproteinaemia. In the case reported by Benirschke *et al.* (1962) a similar double population of cells with abnormal chromosomes is present, as their pictures clearly demonstrate, although the authors failed to notice them. In this case too both type I and II abnormalities existed and two classes of macroglobulin were present.

As macroglobulinaemia is a disease of middle-age it may be suggested that the individual acquires the abnormal lymphoid cell line earlier in life and then some stimulus occurring in later life may be necessary before the cells become functional in the production of macroglobulin. In Nordöy's (1962) case the presence of an increased number of plasma cells and lymphocyte-like cells in the marrow was demonstrated two years before a diagnosis of macroglobulinaemia could be made. In view of recent work concerning the relationship between lymphocytes and protein synthesis (Elves *et al.*, 1963a, 1963b), it is tempting to postulate that this stimulus may be antigenic. On receipt of the stimulus by the abnormal lymphocytes they probably undergo a transformation similar to that observed *in vitro* (Elves and Wilkinson, 1962, 1963; MacKinney *et al.*, 1962) and *in vivo* in rats (Gowans *et al.*, 1961), the end-product being large protein-manufacturing cells. This would explain the recent work in which macroglobulin production has been demonstrated in plasma cells and basophilic reticulum cells (Cohen *et al.*, 1963) or large lymphocytes (Zucker-Franklin *et al.*, 1962) by immunocytochemical techniques. It would be of interest, therefore, to follow the progress of the two younger patients (Cases 2 and 3) over a number of years, as it is possible that they may throw some light upon the natural history of the disease as well as on other aetiological factors. As more cases of this rare disease are studied cytogenetically it may become possible to associate different macroglobulins with the various types of abnormal chromosome.

The origin of these abnormal autosomes presents another problem. German *et al.* (1961) suggested that the abnormal chromosome in their patients, a type I, was an isochromosome for the long arm of a group 2 chromosome. Patau (1961), agreeing with this hypothesis, suggested mechanisms involving pericentric inversion, centromere misdivision, and mitotic non-disjunction, by which both type I abnormalities and the chromosome described by Bottura *et al.* (1961) may be produced.

Whereas the type I abnormality in the above cases may be produced by such mechanisms, the type II chromosome is not so easily explained. As Patau (1961) pointed out, it is difficult to accept that these genetic abnormalities which produce similar biochemical lesions are derived from different chromosomes, and it would therefore be desirable to find a mechanism by which type II abnormality may be derived from a No. 2 chromosome. In some cells this chromosome appears to be lighter-staining than other members of the karyotype (see Fig. 2), and this leads to the suggestion that some degree of "under coiling" exists

and so direct comparison with normal chromosomes may not be valid. Arm ratios of the abnormal type II chromosomes in Cases 2 and 3 were found in this laboratory to be 1.7-2.0 and arm ratios for No. 2 chromosomes range from 1.4 to 2.0, indicating a morphological similarity. The chromosome may have been produced by a defect in the coiling mechanism during mitosis, which is followed by and may even have caused mitotic non-disjunction. The origin of these chromosomes is, however, likely to be a matter for argument for years to come.

If non-disjunction is involved in the production of these abnormal karyotypes one would expect to find a cell-line deficient in a No. 2 chromosome, but as yet no such cell has been found in these patients. Patau (1961) explained this absence by suggesting that such cells would be non-viable. While this may be true, an alternative explanation may also be advanced which is based upon the implied presence of genes controlling γ -globulin synthesis upon the long arm of a No. 2 chromosome. A cell with such a deficiency would probably be less well able to synthesize this globulin and may therefore be immunologically incompetent. As the lymphocyte culture technique is probably dependent upon an immune response this particular abnormal cell-type may exist but not be represented in the mitotic population.

One important consequence of studies such as this is that macroglobulinaemia has clearly been shown to be a disease distinct from other disorders of the reticulo-endothelial system. This is particularly true of myelomatosis, in which a similar morphological picture may be found (Martin, 1960) but for which there is no gross chromosomal basis.

Summary

Two types of abnormal large chromosome have been found in 4-8% of the cells in lymphocyte cultures from one patient with macroglobulinaemia and two younger patients with less-marked dysproteinaemia. It is suggested that this chromosome may confer upon the cell the ability to produce abnormal γ -globulin should an appropriate stimulus be received. It is also suggested that the individual may acquire the genetic defect some years before macroglobulinaemia develops. The mechanism by which such abnormal karyotypes arise is not clear, but some possibilities are discussed.

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