Normal and pathological serum levels of α_2 -macroglobulins in men and mice

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SYNOPSIS The serum levels of α_2 -macroglobulin have been measured in normal men and mice and in a number of immunopathological conditions. Normal human concentrations are high in youth, reach their minimum in middle age, and gradually increase with old age. In all age groups the mean is higher in the female than in the male. Conversely, in normal mice the α_2 M level is low in youth, maximum in middle age, and shows a slight depression with old age, and the levels are frequently higher in males than in females; there are also strain variations.

In human immunopathological conditions, there are some deviations from the normal α_2 M level but these are seen to be changes from the normal distribution of values around the mean, rather than significant elevation or depression of mean values. In some disease states studied there are differences between the sexes in the deviation from normal.

'Abnormal' strains of mice had α_2 M levels within the range exhibited by 'normal' mice but changes in the levels are seen in mice with various myelomas.

In recent years there have been reports indicating an association between α_2 -macroglobulin (α_2 M, MW 820 000) and the development and function of lymphocytes (McNeill, 1970; Havemann, Dosch, and Bürger, 1970; Chase, 1972; Ford, Caspary, and Shenton, 1973). There are also indications that it is present on the surface of a subpopulation of lymphocytes (Sell, 1970; McCormick, Nelson, Tunstall, and James, 1973; James, Tunstall, Parker, and McCormick, 1974) and may be synthesized by peripheral blood lymphocytes (van Furth, Shuit, and Hijmans, 1966; Tunstall and James, 1974). It was therefore thought important to establish normal serum levels of α_2 M in men, over which there has been some controversy (Adham, Wilding, Mehl, and Haverback, 1968), and in mice, and to look at levels in various pathological states with emphasis on those involving disturbances of the immune status.

Materials and Methods

PREPARATION OF ANTISERA

Antisera to human and mouse α_2 M were produced in New Zealand white rabbits by injection of purified preparations of α_2 M. The human α_2 M was isolated by repeated zonal ultracentrifugation of Cohn

fraction III O (kindly given by Ortho Pharmaceuticals, Raritan, New Jersey) and the mouse α_2 M by starch block electrophoresis of the 19S G200 Sephadex fraction of normal mouse serum. On days 0 and 21 the α_2 M (2 mg in Freund's complete adjuvant) was given intramuscularly into three or four sites, and a third injection (2 mg in alum) was given intraperitoneally on day 42. The rabbits were bled on day 49 and at three to four week intervals thereafter without further challenge. The antisera were rendered monospecific for α_2 M by absorption; the antihuman α_2 M was absorbed with the low molecular weight ultracentrifuge fraction of Cohn III-O, the antimouse α_2 M with the 7S fraction of normal mouse serum (obtained by G200 Sephadex gel filtration).

ESTIMATION OF α_2 M

Serum α_2 M was measured by a standard Mancini gel diffusion method. Standard antigen in four different concentrations was included on every plate. The standard for the human measurement was a human α_2 -macroglobulin reference obtained from Meloy Laboratories Inc (Springfield, Virginia, USA). The mouse standard consisted of a pool of normal serum, 25 μ l from each of 40 female A/HeJ mice aged between 5 and 6 months.

The human measurements are given in milligrams

of α_2 M per dl whereas those for mice are given as a percentage of the standard serum. Serum samples from mice were diluted 1 in 2 with buffer before application to the plates.

SERUM SAMPLES

Normal human serum samples were obtained from healthy donors, aged 18-70, from the Blood Transfusion Service, Royal Infirmary, Edinburgh. Approximately 20 samples per decade for each sex were tested.

Table I shows the human pathological sera tested, giving the diagnosis and numbers of patients.

Disease	No. of Cases	
	♂	♀
Chronic lymphatic leukaemia	23	8
Acute lymphatic leukaemia	2	1
Chronic myeloid leukaemia	5	—
Acute myeloid leukaemia	6	4
Lymphosarcoma	12	15
Reticulum cell sarcoma	4	2
Hodgkin's disease	17	16
Myeloma	13	16
Macroglobulinaemia	5	2
Melanoma	4	12
Pemphigoid	1	5
Mycosis fungoides	4	2
Dermatitis herpetiformis	14	9
Bronchogenic carcinoma	17	1
Breast carcinoma	—	8

Table I Clinical diagnoses of patients

Individual mouse serum samples were obtained by killing under ether and bleeding from the inferior vena cava. Ten strains of mice were tested at the ages of 6-8 weeks, 12-16 weeks, and 1 year and over. Approximately 20 mice of each sex in each age group were tested. A/HeJ, Balb/c, CBA, C₅₇Bl, and C₃H mice were obtained by brother-sister mating of breeding pairs originally obtained from sources described in a previous paper from this laboratory (James and Milne, 1972). (CBA × A)F₁ hybrids were bred from this stock. AKR mice and NZB mice were also bred in this department from stock supplied by the Zoology Department, University of Edinburgh, and the Immunology Department, Middlesex Hospital Medical School, London, respectively. Old AKR mice have a tendency to develop thymomas and NZB mice to develop autoimmune disease. Athymic nu/nu mice were purchased from the Medical Research Council Laboratory Animal Centre (Carshalton, England), and Schneider Swiss mice, reared under specific pathogen-free conditions, were obtained from the Animal Diseases Research Organization (Moredun Institute, Edinburgh).

α_2 M serum levels were also measured in Balb/c

mice bearing the following myelomas, IgG₁ (MPC 25), IgG_{2a} (ADJPC5), IgM (MOPC 104EM), and IgA (MOPC 47a).

PRESENTATION OF RESULTS

The α_2 M values of groups of normal men and mice are expressed as arithmetic means $\pm$ 2 standard errors. Due to age and sex variations, and the wide individual variations, the levels of α_2 M in pathological sera have been plotted individually, and comments will be made on those which are appreciably elevated or depressed: the arithmetic mean $\pm$ 2 standard errors of normal sera are represented as shaded areas on the figures showing the pathological levels.

Results

HUMAN α_2 -MACROGLOBULIN LEVELS

Normal human serum levels of α_2 M for each sex are shown in figure 1. The results are expressed as the arithmetic mean $\pm$ 2 standard errors for donors from each decade. It should be noted that over this age range, males had lower levels than females. Levels of α_2 M in cord blood and young children have been found to be very high (James, Johnson, and Fudenberg, 1966; Ganrot and Scherstén, 1966) and to fall fairly rapidly from 15 to 20 and continue to decline more gradually until the age of 30 to 40. A similar decline in α_2 M levels was found in these studies; however, instead of reaching a plateau level, the levels in both sexes appeared to be rising in the higher age groups. Samples from normal individuals over 70 years of age were not tested. It is reasonable to conclude from these results that both the age and sex of the patient have to be taken into account when assessing levels of α_2 M in pathological sera.

The levels of α_2 M in the sera of leukaemic patients are shown in figure 2. The largest group were those

Fig 1 Normal human α_2 M serum levels (arithmetic means $\pm$ 2 standard errors)

Fig 2 Human α_2 M serum levels in patients with leukaemia
In figs 2-5 the shaded areas represent the arithmetic mean of normal serum α_2 M levels, ± 2 standard errors

with chronic lymphatic leukaemia and of these, 11 out of 23 males and two out of six females had depressed α_2 M levels whereas five males and four females had elevated levels. In the case of the patients with acute myeloid leukaemia, five out of the six males and two out of the four females had depressed levels. In the chronic myeloid leukaemia and acute

lymphatic leukaemia cases there were too few patients to establish any trend.

Fifty per cent of the patients with Hodgkin's disease had elevated levels of serum α_2 M whilst those with lymphosarcoma showed a bias towards depressed levels. All the six patients with reticulum cell sarcoma had elevated levels (fig 3). There

Fig 3 Human α_2 M serum levels in patients with sarcomas

Fig 4 Human α_2M serum levels in myeloma or macroglobulinaemia patients

Myelomas; ● IgG, ○ IgA, △ IgM, □ IgD, ⊗ light chain

was no apparent correlation between the α_2M serum level and the stage of the Hodgkin's disease.

The serum levels of patients with macroglobulinaemia and various myelomas are shown in figure 4. Of the patients with IgG myelomas, 50% had depressed α_2M levels, but the sex distribution was uneven, more females than males having low levels. The two light chain myelomas and two IgD mye-

lomas tested also had depressed α_2M , but of the three IgM myelomas one was normal, one elevated, and one depressed. The IgA myelomas appeared to be associated with a normal or elevated level of α_2M . In general the patients with macroglobulinaemia had depressed α_2M levels.

Sera from a number of other pathological conditions were also tested for α_2M , including non-

Fig 5 Human α_2M levels in non-malignant diseases of possible immunological aetiology, and in malignancies of tissues other than those of immunological systems

Fig 6 Normal α_2 M levels in mice (arithmetic means $\pm$ 2 standard errors)
Age of mice:
□ 6-8 weeks, ▨ 3-4 months, ■ over 1 year.

Fig 7 α_2 M serum levels in Balb/c mice with myelomas
The shaded areas represent the arithmetic mean $\pm$ 2 standard errors of age-matched normal Balb/c Mice

malignant diseases with a possible immunological aetiology, and malignancies of non-lymphoid tissues, and the results are shown in figure 5. In all of these conditions there appears to be a fairly even distribution above and below normal levels.

The α_2 M serum levels in normal mice are shown in figure 6. In general, young mice had the lowest level and the highest levels were reached either in middle or old age. Male mice frequently had higher levels than female mice of the same age and strain.

The levels of some abnormal mouse strains are shown in table II and all these levels, with one exception, where the mice were only 5 weeks old, fell within the range of levels covered by the normal strains. The levels in myeloma-bearing Balb/c mice compared with age- and sex-matched normals are shown in figure 7. It is apparent that IgA, IgM, and IgG_{2a} myelomas caused the α_2 M level to be depressed whereas in the case of IgG myelomas, the level was normal in males and elevated in females.

Strain	Abnormality	α_2 M Standard Serum (%) ¹					
		6-8 Weeks		3-4 Months		Over 1 Year	
		♂	♀	♂	♀	♂	♀
AKR	High incidence of spontaneous thymomas in aged mice	127 $\pm$ 6	123 $\pm$ 3	122 $\pm$ 8		114 $\pm$ 4	
Nu/nu	Congenitally athymic	109 $\pm$ 8	86 $\pm$ 4				
Schneider Swiss	Specific pathogen free	77 $\pm$ 4*	101 $\pm$ 2			123 $\pm$ 12	
NZB	Develop autoimmune haemolytic anaemia						132 $\pm$ 6

Table II Some α_2 M serum levels of 'abnormal' strains of mice

¹Values given are arithmetic means $\pm$ 2 standard errors.
*These mice were 5 weeks old.

Discussion

The normal human serum levels of α_2 M reported here agreed with those found by James *et al* (1966) and, in addition, the age and sex differences found confirmed the findings of Ganrot and Scherstén (1967) and other workers (James *et al*, 1966; Housley, 1968).

Elevation of the α_2 M globulin fraction of serum is common in patients with various malignant and non-malignant disorders (Sunderman and Sunderman, 1964), but in many of these reports there has been no differentiation between low molecular weight α_2 globulins and α_2 -macroglobulins. Few measurements of α_2 M have been carried out on patients with immunological disorders; however, in those who were studied (James *et al.*, 1966; Housley, 1968) a number of changes were found, and the variations from normal were found to be dependent on the sex in certain disorders.

In the present study of immunopathological states, the mean α_2 M levels were not significantly different from normals as there was wide variability in individual levels. However, in several states the distribution of the individual α_2 M levels did not follow the normal pattern. For example, in chronic lymphatic leukaemia the distribution of α_2 M levels was below the normal mean in males, and above in females, and in acute myeloid leukaemia both sexes had a distribution lower than normal. In the cases of sarcomas studied, those with reticulum cell sarcoma had elevated levels, those with lymphosarcoma tended to have depressed levels, whilst those with Hodgkin's disease, a sarcoma of mixed lymphocytic and reticulocytic type, 50% of the levels were above the normal mean. In a number of malignancies that did not directly involve immune systems, the distribution of α_2 M levels was normal.

It is interesting to note from the results of the measurements on mouse sera that the changes in concentration with age and sex appeared to be the reverse of those found in man, results similar to those of Ganrot (1968) with rat and ox sera. There were significant differences in α_2 M levels between various strains of mice, but these did not correlate with immunologically high or low responders. The 'abnormal' strains had levels within the range covered by the more 'normal' strains.

Another difference between man and mouse was seen in the myelomas. In man the α_2 M levels in IgG myelomas were depressed, whereas in mouse they were normal (male) or elevated (female), and, where in men with IgA myelomas the level was elevated, in mice it was depressed. Because of these differences between men and mice, caution must be

observed in extrapolating from experimental mouse models to the human situation.

These measurements of serum α_2 M levels do not help to elucidate the precise role of α_2 M, but they do indicate that in disturbances of the immune system and in the cells that are involved in the immune response, there are perturbations in the α_2 M levels. It still remains to be established whether α_2 M levels could be of help in diagnosis or prognosis, and whether they are influenced by therapy, and further studies on these aspects are being carried out in this laboratory.

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