incubated at 37 °C in a 5% CO_2 and 95% humidified air environment for 7 days. Proliferation was evaluated by adding 1 μCi/well of H^3TdR (sp. act. 50 mCi/ml) 18 h before harvesting.

Anti-Ia monoclonal antibodies were produced as previously reported[19]. Briefly, the hybridoma were obtained by fusing spleen cells from Balb/c mice, immunized with membrane-enriched fraction from the Daudi human lymphoblastoid cell line, with the mouse myeloma P3-X63Ag8 (D1-12 hybridoma) and P3-NS1Ag4 (D4-22 hybridoma). In studies of the effect of adding anti-Ia monoclonal antibodies to MLR, 50 μl of either D1-12 or D4-22 hybridoma culture fluid were added at the onset of the MLR.

The MLR proliferative response was of the same order of magnitude in MM and in MGUS as in normal controls. This result was the same both when pathological lymphocytes were used as responding cells (table 1) or as stimulating cells (table 2).

Moreover, the addition of either one of the monoclonal anti-Ia antibodies at the onset of the MLR resulted in more than 80% of inhibition of the proliferative response. In contrast, culture fluids from the parental myeloma PX63 had no inhibitory effect.

Although the biological significance of the MLR is not completely determined, it is evident that it reflects an important role played in vivo by the immune system[12, 13, 20]. An impaired MLR has been demonstrated by several workers in diseases characterized by immunodeficiencies, as Hodgkin's disease[21]. By analogy, in MM where the immunodeficiency is well-known, and B and T cell populations are involved in the disease[5-11], similar impairment would be expected. However, our data show proliferative values in the normal range when pathological cells are used as either responding or stimulating cells.

Moreover, when pathological lymphocytes were used as stimulating cells, a normal inhibition of the MLR by monoclonal anti-Ia antibodies was obtained. Since the MLR proliferative response can be abrogated by anti-Ia antibodies in MM and in MGUS as in normal controls, we suggest that the expression of Ia molecules on the surface of pathological lymphocytes is not altered and also the immunological mechanisms responsible for this inhibition are functionally normal.

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Antibodies from patients with liver diseases and from normal human or animal sera against glutaraldehyde-polymerized albumins: lack of species specificity

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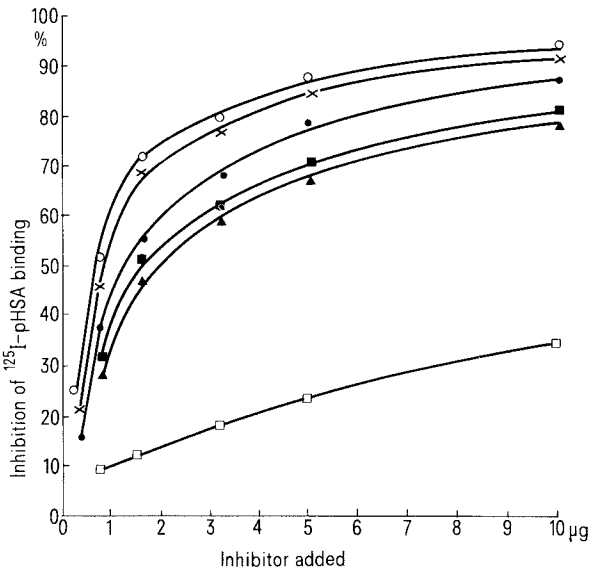
Summary. By the use of glutaraldehyde-polymerized albumins of different species (human, rabbit, bovine, goat and mouse) it was demonstrated that anti-albumin antibodies in sera of patients with liver diseases and in normal human and animal (rabbit, mouse) sera are not species specific.

Antibodies against glutaraldehyde(GA)-polymerized human serum albumin have been described in patients with different liver diseases[2-6], and have been shown to be specific for the new antigenic determinants induced in albumin by GA treatment[7]. Anti-albumin antibodies (AAA) have also been found, in low concentrations, in normal human[8], mouse[9] and rabbit[10] sera. Unlike the receptor for polymerized albumin on hepatitis B surface antigen(HBsAg)-carrying particles, and the Clq component of complement, both of which bind polymerized human serum albumin in a species-restricted manner[11,12], AAA from pathological sera seem to react with a non-species-specific determinant on the polymerized human serum albumin molecule[13]. In this paper we report results which substantiate the latter supposition, bringing quantitative arguments that AAA from different species are not species specific.

Material and methods. Human serum albumin (HSA), rabbit serum albumin (RSA) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO,

USA. Goat serum albumin (GSA) and mouse serum albumin (MSA) were isolated from serum by preparative electrophoresis in agarose gel[14] and their purity was ascertained by electrophoresis. GA-polymerized albumins corresponding to a protein/GA weight ratio of 8/1 were prepared as previously described[7]. Since electrophoresis in 6% polyacrylamide gel[15] showed that GA-treated albumin preparations present a different content in polymeric and monomeric fractions, the preparations were chromatographed on a Sephacryl S-200 column (123 × 3.0 cm) equilibrated with 0.05 M Tris-HCl buffer, pH 8.0 containing 0.25 M NaCl. The leading polymeric albumin peaks were pooled. The polymers were called pHSA, pRSA, pBSA, pGSA and pMSA. Radioactive GA-polymerized HSA (^{125}I-pHSA) was obtained from HSA previously labeled with carrier-free Na ^{125}I (100 mCi/ml) (Radiochemical Centre, Amersham, U.K.) at 0.2 mCi/mg protein by the lactoperoxidase technique[16]. The specific radioactivity of ^{125}I-pHSA was 10^5 cpm/μg protein.



Inhibition of ^{125}I-pHSA binding to AAA in serum from a patient with acute viral hepatitis by different amounts of inhibitors: pBSA (○—○), pRSA (x—x), pGSA (●—●), pHSA (■—■), pMSA (▲—▲) and pHRP (□—□). Reaction tubes contained 0.25 ml of a given dilution of pathologic serum (previously found to bind 50% of the ^{125}I-pHSA in the absence of any inhibitor) and 0.25 ml of inhibitor in various concentrations. After 30 min incubation at 37 °C, 0.25 ml of ^{125}I-pHSA (0.3 μg) were added. All the dilutions of ^{125}I-pHSA, of inhibitor or of pathologic serum were made in barbital buffer, pH 8.6, μ = 0.05. After overnight incubation at 4 °C, a suspension of *Staphylococcus aureus* (strain Cowan I) in barbital buffer (0.25 ml containing 10^9 bacteria) was added, then incubated for 30 min at room temperature and sedimented. ^{125}I-pHSA in each pellet washed once with ice-cold barbital buffer, containing 0.1% ovalbumin, was determined in a well-type γ-counter. Controls included tubes without inhibitor (binding control) and tubes with 0.1% ovalbumin in barbital buffer instead of pathologic serum (nonspecific binding control). All assays were carried out in duplicate. The percent inhibition was calculated according to the formula[20]:

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100,$$

in which A is the percent binding in the absence of inhibitor and B is the percent binding in the presence of inhibitor. The results were plotted as the percent inhibition vs the amount of inhibitor used (μg).

Horseradish peroxidase (HRP), type II (Sigma Chemical Co., St. Louis, MO, USA) was treated with GA as shown for albumins (protein/GA weight ratio = 8/1). The resulting polymer was called pHRP.

High AAA titer human sera were obtained from patients with acute viral hepatitis (5 cases), chronic hepatitis (5 cases) and liver cirrhosis (2 cases). Normal human sera were obtained from healthy blood donors. AAA in pathologic and normal human sera were determined by radioimmunoassay[8] using a protein A containing *Staphylococcus aureus* (SA) suspension (strain Cowan 1). The serum to be tested was mixed with ^{125}I-pHSA and the resulting immune complex was adsorbed on the bacteria. The AAA level was expressed by the ^{125}I-pHSA binding capacity of serum at a dilution of 1:160, i.e. the percentage of the added radioactivity bound to the SA suspension. The means and standard deviations (SD) of the ^{125}I-pHSA binding capacity in human sera were 64.2 ± 8.8% for pathologic sera (12 cases) and 17.0 ± 2.0% for normal sera (3 cases) at a serum dilution of 1:160 and using 0.3 μg ^{125}I-pHSA. AAA in normal mouse sera (CBA strain) were detected by radioimmunoassay with ^{125}I-pHSA[9]. Normal mouse sera (3 cases) bound 20.3 ± 1.5% of ^{125}I-pHSA (0.5 μg) at a serum dilution of 1:160. AAA in normal rabbit sera (Chincilla) were detected by passive hemagglutination of sheep erythrocytes coated with pRSA (E-pRSA)[10]. The mean $\log_2$ hemagglutination titer of normal rabbit sera was 5.3 ± 0.6 (3 cases). The specificity of human and mouse AAA was tested by inhibition radioimmunoassay[7] and of rabbit AAA by inhibition of passive hemagglutination[10].

Results and discussion. The inhibition curves shown in the figure demonstrate that in serum of patients with liver diseases the reaction between AAA and ^{125}I-pHSA was inhibited not only by polymeric human albumin but also by polymeric albumins of different species (bovine, rabbit, goat and mouse). GA-polymerized peroxidase (pHRP) used as a control, showed a weak inhibitory capacity in comparison with the polymeric albumins. From the inhibition curves the amount of inhibitor for 50% inhibition was determined. The means ± SD of inhibitors in μg required for 50% inhibition in 12 sera from patients with liver diseases were: pBSA, 0.8 ± 0.3; pRSA, 0.9 ± 0.3; pGSA,

Reaction of rabbit and mouse AAA with homologous and heterologous polymeric albumins as determined by inhibition of passive hemagglutination and by inhibition radioimmunoassay

Inhibitors	Inhibition of the reaction between rabbit AAA and E-pRSA[a]		Inhibition of the reaction between mouse AAA and ^{125}I-pHSA[c]	
	μg/ml	Weight ratio[b] for maximum inhibition	μg for 50% inhibition	Weight ratio[d] for 50% inhibition
pBSA	0.03 ± 0.02	0.08	ND	ND
pHSA	0.16 ± 0.07	0.40	1.8 ± 0.1	0.72
pRSA	0.40 ± 0.14	1.00	ND	ND
pMSA	0.50 ± 0.17	1.25	2.5 ± 0.2	1.00

[a] The inhibitory effect of homologous and heterologous polymeric albumins on the reaction between rabbit serum and E-pRSA was tested using the last but one serum dilution giving positive hemagglutination and serial inhibitor dilutions. The means ± SD of the lowest inhibiting concentrations for 3 rabbit sera are presented. [b] Mean weight ratio of inhibitor to pRSA for maximum inhibition of passive hemagglutination. [c] Inhibition radioimmunoassay was performed as described in the figure by using the normal mouse serum dilution which binds 50% of the ^{125}I-pHSA in the absence of any inhibitor. The means ± SD of inhibitor amounts for 3 mouse sera are presented. [d] Mean weight ratio of inhibitor to pMSA in the assays with 50% inhibition. ND, not determined.

1.3±0.4; pHSA, 1.7±0.6; pMSA, 1.8±0.6 and pHRP 27.0±7.9. The mean weight ratios of heterologous polymeric albumins to homologous pHSA, determined for a 50% inhibition (0.47 for pBSA, 0.53 for pRSA, 0.76 for pGSA and 1.06 for pMSA) as well as the complete radioimmunoassay inhibition curves (fig.) proved that pBSA, pRSA and pGSA are better inhibitors than pHSA, while pMSA inhibitory capacity is close to that of pHSA. The inhibitory capacity of pHRP was 15–34 times weaker than that of polymeric albumins, as can be seen from the mean weight ratios of pHRP to polymeric albumins for 50% inhibition. Similar results were obtained for AAA found in normal human sera. Thus the means ±SD of inhibitors in µg required for 50% inhibition in 3 normal human sera were: pBSA, 1.4±0.5; pRSA, 1.6±0.5; pGSA, 1.4±0.3; pHSA, 2.3±1.3, pMSA, 2.4±1.4 and pHRP, 34.0±4.3.

In the table are given the results of the inhibition experiments performed with normal rabbit and mouse AAA. The weight ratios of heterologous polymeric albumins to homologous pRSA at maximum inhibition of passive hemagglutination showed that rabbit AAA react better with pBSA and pHSA than pRSA. The reacting capacity of rabbit AAA with pMSA was comparable with that shown with pRSA. In the mouse system heterologous pHSA showed a stronger inhibitory capacity than homologous pMSA on the reaction between mouse AAA and ^{125}I-pHSA.

The similar, or even stronger, AAA binding activity shown by heterologous vs homologous polymeric albumins demonstrate the lack of species specificity for AAA in both pathologic and normal human and animal sera. Thus our results extend the findings of Thung and Gerber[13] who showed that AAA in pathologic sera react with both polymeric homologous human albumin and heterologous bovine and rat albumin. The lack of species specificity has also been demonstrated for AAA elicited in rabbits by immunization with GA-polymerized rabbit albumin[17] and not for rabbit and mouse antibodies elicited against GA-polymerized immunoglobulin[18]. The capacity of GA-treated heterologous albumins to react with AAA shows that GA treatment produces similar antigenic determinants in albumins of different species. GA-treated peroxidase, used as a control, inhibited, to some extent, the reaction between AAA and pHSA, thus suggesting that pHSA and pHRP have some common antigenic determinants. It is reasonable to assume that after GA treatment albumin and peroxidase share mainly the chemical groups which appear following the reaction of GA with the ε-amino groups of the lysine residues[19], and not the antigenic determinants dependent on the polymerization process, since the latter are probably related to the particular amino acid environment of each protein[7]. This supposition could explain the high capacity of GA-polymerized albumins to react with AAA from different species and the weak reacting capacity of the GA-polymerized peroxidase not related to serum albumin.

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Phagocytosis in diabetic subjects: increase in hydrophobicity of granulocyte cytoplasmic membrane

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Summary. Granulocytes from diabetic subjects have impaired ability to engulf bacteria; the data obtained suggest that the alterations are correlated with an increase in surface hydrophobicity, as measured by contact angle.

Subjects with diabetes mellitus have altered lipid-metabolism, which is reflected in the cellular membrane. An increase in microviscosity, that is to say, a decrease in fluidity, of the erythrocyte membrane was revealed by analyses using fluorescence-depolarization[1]. The reduced membrane fluidity is apparently related to an increase in the molar ratio of cholesterol-to-phospholipids (C/PL)[1,2]. Alterations in the C/PL ratio must give rise to changes in