Receptors for polymerized albumin on liver cells

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Summary. By the use of rabbit polymerized albumin labelled with fluorescein isothiocyanate, or coated on sheep red blood cells, specific receptors on rabbit liver cells were demonstrated. The possible biological role of these receptors is discussed.

A new type of autoantibodies directed against polymerized albumin was described in some patients with liver dysfunction[2-4] and in rabbits with hepatic disorders[5]. The albumin polymers are prepared either by spontaneous ageing of albumin solution or by its cross linking with glutaraldehyde[4]. Albumin polymers are formed in vivo too, as suggested by their detection in some pathological sera[2,6]. We have recently suggested[4] that the removal of the albumin polymers from the circulation may be performed by specific receptors on liver cells. We report here some evidence supporting this hypothesis.

Material and methods. The hepatocytes were prepared from rabbit liver according to Hopf et al.[7]. The viability of hepatocytes exceeded 90% (phase contrast microscopy). The cell suspension contained less than 1% of Kupffer cells. Rabbit albumin (RSA) was isolated from serum by preparative electrophoresis in agarose gel[8] and found to be pure by electrophoresis. Glutaraldehyde treatment of RSA[3] lead to the formation of a soluble polymer (RSAP) with a molecular weight of 680,000 daltons as determined by gel filtration on Sepharose 6B. Fluorescein isothiocyanate (FITC) conjugates of RSA and RSAP were prepared by reacting 20 mg ml^{-1} protein with 1 mg ml^{-1} FITC for 30 min at 37 °C and overnight at 4 °C, at pH 9.2 in 0.1 M carbonate buffer. The molar FITC/protein ratio was between 6 (RSA) and 9 (RSAP).

Results and discussions. FITC-RSAP stained more than 90% of hepatocytes. The majority of fluorescent hepatocytes presented ring (figure 1C) and patchy (figure 1D) staining. The strongest fluorescence was noticed on small-sized hepatocytes (figure 1A). Very few hepatocytes showed cap formation even at 37 °C. If the cell suspension was incubated for 2 h at 37 °C, many cells exhibited progressive pinocytosis (figures 1B und E). The intensity of fluorescence was sometimes considerably enhanced if the cells had been incubated before staining for 2 h at 37 °C (2×10^6 cells ml^{-1}). This may indicate that the cells have in vivo loosely attached albumin on their surface which is lost during the incubation at 37 °C. No staining was observed with FITC-RSA, even at high doses of the ligand (2 mg 10^7 $cells^{-1}$ (figure 1F)). The staining with FITC-RSAP was not reduced by preliminary incubation of cells with non-fluorescent RSA (1 mg 10^7 $cells^{-1}$) while approximately 70% inhibition of staining was recorded with non-fluorescent RSAP (1 mg 10^7 $cells^{-1}$). No significant staining of rabbit alveolar macrophages or blood lymphocytes was recorded either with FITC-RSA or with FITC-RSAP. No binding of glutaraldehyde polymerized rabbit IgG on rabbit hepatocytes was found (unpublished results). These results show that the binding of FITC-RSAP to hepatocytes is specific.

The ability of liver cells to bind exclusively FITC-RSAP was verified by a rosette-forming technique, using as indicator cells sheep erythrocytes coated with RSAP (EAP). EAP were prepared according to the method previously described for human albumin[4]. Erythrocytes treated with glutaraldehyde (E) were used as control.

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2 R. Lenkei, G. Mota, M. E. Dan and M. Laky, Rev. roum. Biochim. *11*, 271 (1974).
3 R. Lenkei, V. T. Babes, M. E. Dan, A. Mustea and J. Dobre, J. med. Virol. *1*, 29 (1977).
4 R. Lenkei and V. Ghetie, J. Immun. Meth. *16*, 23 (1977).
5 D. Onica, F. Mihalcu, R. Lenkei, M. Gherman and M. Tudor, Infect. immun., in press.
6 Kawai Tadashi, in: Clinical aspects of the plasma proteins, p. 11. Springer Verlag, Berlin-Heidelberg-New York 1973.
7 U. Hopf, K.-H. Mayer zum Büschenfelde, W. Arnold, New Engl. J. Med. *294*, 578 (1976).
8 D. Motet and V. Ghetie, Rev. roum. Biochim. *1*, 175 (1964).

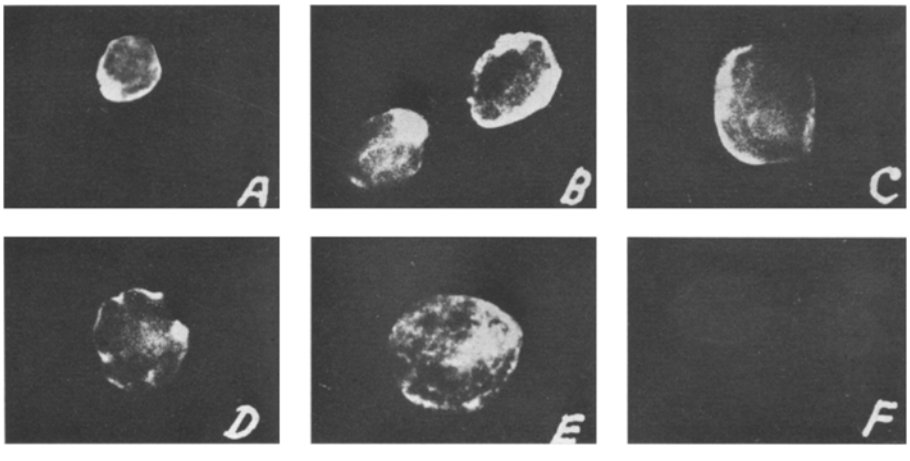

Fig. 1. Fluorescent staining of rabbit hepatocytes with FITC-RSAP (×400). A. Ring staining; B. pinocytosis (early process); C. brocken ring staining; D. patchy staining; E. pinocytosis (late process); F. negative cells (treated with FITC-RSA). To 3–5.10^6 cells, suspended in 0.4 ml TC-199 medium containing 2% bovine serum albumin, 0.1 ml of FITC-RSAP (5 mg ml^{-1}) was added and the mixture was incubated at 4 °C for 45 min. The cells were washed 3 times with cold medium containing or not sodium azide (1 mg ml^{-1}) and finally resuspended in 0.2 ml medium. Cells were examined in suspension under coverslip with ultraviolet light. More than 200 cells were examined and scored in each preparation.

Rabbit hepatocytes formed a significant percentage of rosettes with EAP (48.1 ± 2.1) while with E the cells formed 5 times less rosettes (9.9 ± 0.8). The majority of cells have multiple EAP over the surface or around the membrane (figures 2 A and C). Few cells showed cap-like location of EAP (figure 2B). The cells attaching less than 6 EAP or E were considered as negative (figure 2D).

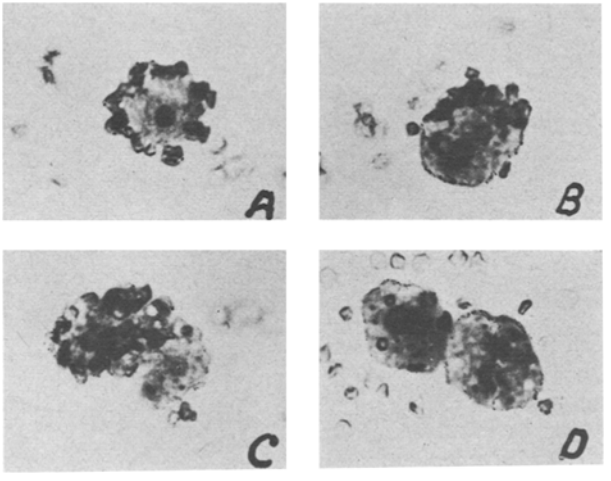

Fig. 2. Photomicrographs of rosettes formed by EAP (×400). A. Cell with ring-like distribution of EAP; B. cell with cap-like distribution of EAP; C. cell with multiple EAP over the cell surface; beneath this cell a negative hepatocyte can be seen; D. 2 negative cells. To 10^6 hepatocytes suspended in 0.1 ml of TC-199 medium containing 2% bovine serum albumin, 0.05 ml of EAP or E (10^9 cells ml^{-1}) was added in a small plastic tube. The mixture was centrifuged at 4 °C for 10 min at 250 g, and further incubated for 1 h at 37 °C. After adding 0.35 ml medium, the deposit was gently resuspended, fixed for 10 min with 0.2 ml 2% buffered formaldehyde and further treated with 0.1 ml 0.2% buffered toluidine blue. After 10 min more than 200 cells were counted.

The lower percent of hepatocytes binding EAP (50%) than that of cells stained with FITC-RSAP (90%) may be due to the different size of EAP (5 μm) and hepatocytes (30 μm), preventing some hepatocytes to form EAP rosettes during centrifugation. Rosette formation between hepatocytes and EAP was inhibited if the cells were previously treated with RSAP. The amount of RSAP which was able to inhibit rosette formation by 50% is lower than 10 μg 10^6 cells^{-1}. No significant inhibition of rosette formation was recorded with RSA (2 mg RSA 10^6 cells^{-1}).

The results presented here seem to show that on rabbit hepatocytes membrane there are receptors specific for the fixation and further manipulation of the polymerized albumin. The lack of the binding of RSA and RSA inability to inhibit the fixation of RSAP, as detected by fluorescence or by rosette formation, suggest that these specific receptors are able to discriminate between the different molecular forms of the albumin. The receptor for albumin on liver cell membrane functions as a binding site for in vivo polymerized albumin conferring to these cells the ability to remove the worn-out polymerized molecules from the circulation of the normal organism. In this way the hepatocytes would be able to select for catabolism the polymerized albumin from the native one. A similar mechanism of IgG catabolism by macrophages was suggested[9], and some evidence that IgG fixation and degradation proceed only after the aggregation of the molecules was presented[10]. The liver damage affects the ability of hepatocytes to remove the polymerized albumin from the circulation, therefore raising its level in serum. The polymerized albumin by its new antigenic sites is potentially able to induce the formation of specific autoantibodies.

9 T. Waldman and V. Ghetie, in: Progress in Immunology, p. 1187. Ed. B. Amos. Academic Press, New York and London 1971.
10 V. Ghetie and L. Buzila, Rev. roum. Biochim. *9*, 125 (1972).

Radiation-released histamine in the rhesus monkey as modified by mast-cell depletion and antihistamine

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Summary. 4000 rads of mixed gamma neutron radiation administered to rhesus monkeys released a significant amount of histamine into their circulation. When the monkeys were treated with a mast-cell histamine depleter (compound 48/80) for 4 days and then irradiated, no increase in circulating histamine was seen. When 48/80 was given 20 min after irradiation, only a slight increase in histamine was seen, indicating that 4000 rads had released most of the mast-cell histamine.

Increased blood histamine levels following ionizing radiation have been reported in rats and man[2-4]. These increased histamine levels appear to correlate well with hypotension[4] as well as with a reduction in the number of tissue mast cells[3]. Because ionizing radiation does cause disruption of mast cells, the suggestion has been made that mast-cell histamine release could be responsible for the observed hypotension[4].

These experiments have been designed to show a) the amount of histamine released by 4000 rads of ionizing radiation, b) the amount of histamine which is blocked from receptor sites by an antihistamine, c) whether the released histamine is of mast-cell or nonmast origin, and d) whether the histamine released could be responsible for the observed hypotension. These experiments were

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2 C. E. Lasser and K. W. Stenstrom, Am. J. Roentg. *72*, 985 (1954).
3 H. A. S. Van den Brenk, Br. J. exp. Path. *39*, 356 (1958).
4 R. P. Webber and F. R. Steggerda, Proc. Soc. exp. Biol. (N. Y.) *70*, 261 (1949).