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Serum inhibitory activity on granulocyte-macrophage colony formation

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Summary. Normal and chloroform-extracted human sera, fractionated by Sephadex column chromatography, have been tested for inhibitory activity on granulocyte-macrophage (GM) colony formation. It was found that this activity is connected with lipoproteins (inhibitors of granulocyte-macrophage colony stimulating factor) and low molecular weight substances (7000; 13,000) which can act as specific polypeptide chalones.

The proliferation and differentiation of granulocyte and macrophage colony forming cells in agar culture (CFC-c) is under the control of the glycoproteid substance called colony stimulating factor (CSF). Many data clearly confirm that CSF function is intimately linked with the regulation of granulopoiesis[1].

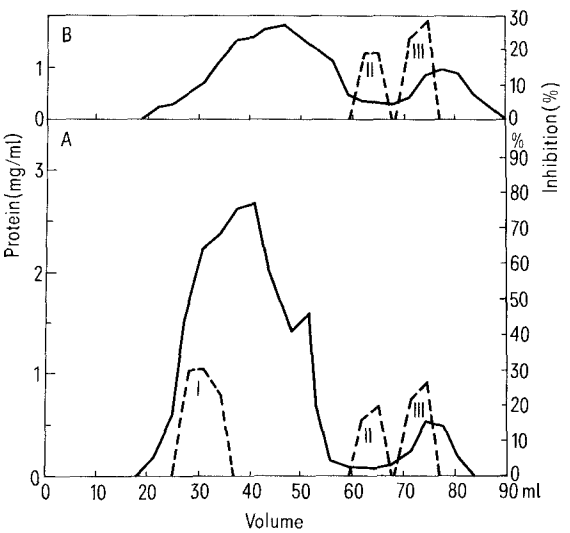
On the other hand, the growth and differentiation of CFC-c can be inhibited by several substances, such as chalones of granulopoiesis which are found in mature granulocytes, and have been precisely characterized by Paukovits et al.[2] as polypeptides with molecular weights ranging from 5000 to 10,000. An inhibitor of CSF biosynthesis has been found by Broxmeyer et al.[3,4], also in mature granulocytes. This inhibitor was characterized as lactoferrin (mol.wt 80,000–100,000, isoelectric points 6.0–6.5), an iron-binding glycoprotein found in the secondary granules, which can suppress the in vitro and in vivo production of granulocyte-macrophage colony stimulating factor by monocytes. The serum unspecific inhibitor of CSF, which has been characterized as a lipoprotein, inhibits the action of CSF directly and nonspecifically[5,6]. My attention has been directed to the study of the inhibition of GM colony formation by normal and chloroform-extracted human sera fractionated by Sephadex column chromatography.

Materials and methods. Chloroform extraction was used in order to remove the lipoproteins from sera[6]. Human sera were pooled from healthy subjects.

Chromatography procedures were carried out on a column (1.6×40 cm) with Sephadex G-200, eluted by 0.9% NaCl; about 100 mg of protein (1.5 ml of serum) was applied to the column. Protein concentration in the fraction samples was determined spectrophotometrically. Molecular weights were determined by chromatography on Sephadex G-200 and G-50.

Colony (> 50 cells/clone) formation was determined by plating 10^5 mouse bone nucleated marrow cells in 1 ml of 0.3% agar medium containing 0.05 ml of fraction sample and 0.05 ml of stimulant (1:2 diluted serum from a mouse treated with *Escherichia coli* endotoxin as an agent enhancing CSF level). Cultures were incubated at 37 °C in a humidified atmosphere containing 7.5% CO_2 in air for 1 week. Colonies and clusters were scored from 3 to 5 plates for each assay point.

Results and discussion. In normal human serum, after the chromatography procedure, the inhibitory activity of granulocyte-macrophage colony formation is distributed between 3 peaks, localized in elution volumes of 30 ml, 64 ml and 73 ml respectively (fig. A). In the chloroform-extracted human serum after chromatography, only 2 peaks were found (fig. B). They were localized in the same elution volumes as peaks II and III in normal serum. I did not find any traces of the activity of peak I in this serum. Molecular weights determined for active substances in each peak of these sera revealed the following data; peak I-200,000, II-13,000, III-7000.



Inhibition of GM colony formation by human sera fractionated by column chromatography on Sephadex G-200. Normal serum (A) and chloroform extracted serum (B). (—) protein; (- - -) inhibitory activity for GM colony formation.

These results should be considered with respect to the present state of knowledge about substances inhibiting proliferation and differentiation of CFC-c. It is known that inhibitors of CSF biosynthesis and chalones of granulopoiesis are produced by mature granulocytes and have been found only in these cells[1]. Up to now, in serum, only the presence of an unspecific lipoprotein inhibitor of CSF has been generally confirmed[5–7].

The experimental system used here precludes any inhibiting action at the level of CSF biosynthesis, and any inhibitors found cannot be described as lactoferrin from granulocytes. The fact of the disappearance of peak I in the serum after chloroform extraction, and its molecular weight (200,000), confirmed the lipoprotein structure of this inhibitory activity[8]. In this situation, I suggest that the inhibitory activity of peaks II and III is due to low molecular weight substances (13,000; 7000) which can act as a polypeptide chalones[2,9,10].

The action of these polypeptides on a GM colony may be unspecific, and we should also take into consideration the fact that human serum was tested on mouse target bone marrow cells and species-differences may be responsible for some of the phenomena. Therefore, the most probable explanation is the releasing of these polypeptides from mature granulocytes into the bloodstream, as specific inhibitors of granulopoiesis.

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Surface topography of granulosa cells accompanied by fragmented oocytes

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Summary. Scanning electron microscopy of granulosa cells (GC) and granulosa cell-like structures (GCLS) revealed that both had lacy foldings, or plicae, on the surface and were identical. The plicae did not always cover the entire surface of GC or GCLS. Both structures were interconnected by multivalent processes.

Granulosa cells surrounding an oocyte extend processes through the zona pellucida to the oocyte, forming gap junctions[2], and via this connection granulosa cells and oocyte continue to interact during their growth and maturation[3–5]. However, when an oocyte shows fragmentation, which is defined as a sign of degeneration[6] leading to elimination, granulosa cells and the processes exhibit concomitant morphological changes. They become larger in size and no longer have a uniformly round shape. The cytoplasm contains more coarse granules and vacuole formation is prominent. Often extremely thick processes of granulosa cells (up to several microns) are observed in photomicroscopy. Granulosa cells often invade the perivitelline space, and on the surface of fragmented oocytes a structure similar to a granulosa cell is observed. Shinohara and Matsuda[7] suggested the possibility that granulosa cells outside the zona pellucida transfer their cytoplasm into the tips of the processes and thus develop the granulosa cell-like structure and ultimately change their position and more into the perivitelline space.

Using scanning electron microscopy, the present research describes the surface morphology of granulosa cells and thickened processes accompanied by fragmented oocytes, which have been described as above on the basis of light microscopy.

Materials and methods. Female Wistar strain rats, aged between 8 and 12 weeks, were used. They were kept in an air-conditioned room at 24 °C and exposed to light from 07.00 h to 19.00 h. Only animals showing regular 4- or 5-day sexual periodicity were supplied for the experiment, without regard to estrus cycles. Bilateral ovaries were extirpated under ether anesthesia and the surrounding connective tissues and blood were removed on a clean filter paper. In a plastic dish containing cold 0.9% NaCl the ovaries were randomly punctured in order to liberate the ovarian oocytes. Under a stereoscopic microscope, fragmented oocytes were selectively collected by a mouth-controlled micropipet, and fixed in 2.0% glutaraldehyde in 0.05 M Sorensen's phosphate buffer solution (SPBS), pH 7.4, for 2 h. After a brief rinse in 0.15 M SPBS, the zona pellucida of the oocytes was removed in 0.15 M SPBS containing 0.1% trypsin, at 25 °C, without separation of the granulosa cells from the oocytes. Usually 3–6 min of the enzymic treatment was sufficient for obtaining zona-free and granulosa cell-attached oocytes. Rinsing again, the oocytes were attached to a coverslip coated with 0.2% poly-L-lysine[8]. Following dehydration in a graded series of ethanol-distilled water solutions from 30 to 100%, the specimen was infiltrated with isoamyl acetate. The oocytes were critically point-dried in liquid-gaseous CO_2, and gold sputter-coated about 300 Å in thickness. A scanning electron microscope (Super 3A: ISI-Akashi) was used for observation of the surface topography.

Results. Granulosa cells (GC) outside the zona pellucida, and granulosa cell like structures (GCLS) in the perivitelline space had a common surface architecture; lacy foldings, or coarse plicae, against a relatively smooth background surface. Occasionally, GCLS in the perivitelline