RECEPTOR DIFFERENCES BETWEEN RESIDENT AND PEPTONE-STIMULATED PERITONEAL MACROPHAGES REVEALED BY SALMOSAN

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There is much evidence that complex carbohydrate structures located on cell membranes can play an important role in biological recognition. The external location of the carbohydrate components reflects a general principle of structure of cell membranes. Interaction between sugar molecules which are components of the cell wall and receptors recognizing these sugars is essential for intercellular contact, taking place during interaction both between different cells and between the body cells and microorganisms [1, 5, 11]. In the early stages of infection, during recognition of the microorganism, the abundance of sugar-specific (lectin-like) receptors, with which the mechanism of attachment of nonopsoninized microorganisms is connected [10], being an essential part of the process of phagocytosis, is particularly important. But the abundance of these receptors on the surface of macrophages belonging to different populations may itself differ. That is why they have different roles in the process of recognition and presentation of an antigen. Lectin-like receptors are usually studied by utilizing the effect of inhibition of attachment of foreign cells to the phagocyte membrane under the influence of mono- or polysaccharides [6, 7].

The aim of this investigation was to study the degree of abundance of lectin-like receptors on the surface of resident (RPM) and peptone-stimulated (PSPM) peritoneal macrophages, using salmosan as the polysaccharide.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 16-18 g. Depending on the experimental condition the mice were given an intraperitoneal injection of labeled sheep's red blood cells (SRBC-^{51}Cr), unlabeled sheep's red blood cells (SRBC), SRBC-^{51}Cr simultaneously with salmosan, or SRBC-^{51}Cr sensitized by salmosan, 60 min before peritoneal exudate cells (PEC) were obtained. Uptake of SRBC-^{51}Cr was determined after the above procedures [3].

PEC were obtained from normal mice, without injection of an irritant (RPM population) or on the 4th day after intraperitoneal injection of 2 ml of nutrient broth (PSPM population). A monolayer of macrophages was obtained by culturing PEC in medium 199 with heparin for 2 h at 37°C, followed by thorough and repeated washing to remove nonadherent cells.

SRBC-^{51}Cr were sensitized with salmosan at 4°C for 18 h. A 10% suspension of SRBC, in a volume of 5 ml, was incubated with 1 mg salmosan, dissolved in 1 ml of physiological saline. After incubation the SRBC were washed with physiological saline and injected in a dose of $3.5 \cdot 10^8$ cells per mouse.

EXPERIMENTAL RESULTS

In the experiment of series I the effect of salmosan was studied on uptake of SRBC-^{51}Cr by RPM and PSPM populations. The mice were given an intraperitoneal injection of SRBC-^{51}Cr together with salmosan or of SRBC-^{51}Cr sensitized by salmosan. Injection of salmosan into mice simultaneously with SRBC-^{51}Cr was shown to reduce the uptake of red cells in the RPM population. A significant reduction of uptake also was found when mice were injected with salmosan-treated SRBC-^{51}Cr (Fig. 1a). Unlike RPM, the phagocytic function of the PSPM population was unchanged both by simultaneous injection of SRBC-^{51}Cr and salmosan and by

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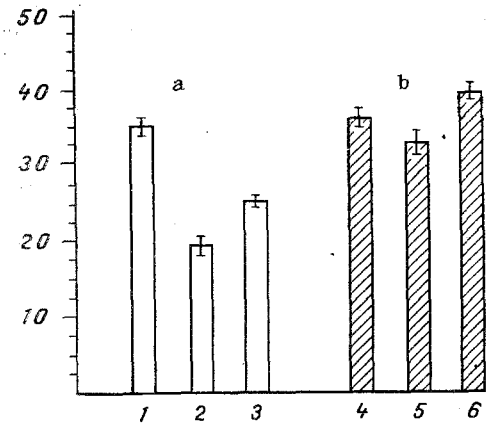

Fig. 1

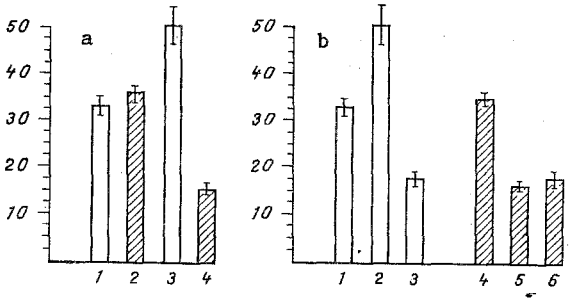

Fig. 2

Fig. 1. Uptake of salmosan-sensitized SRBC-^{51}Cr by RPM (unshaded columns) and PSPM (shaded columns) populations. 1, 4) SRBC-^{51}Cr; 2, 5) salmosan together with SRBC-^{51}Cr; 3, 6) salmosan-sensitized SRBC-^{51}Cr. Here and in Fig. 2, vertical axis – uptake of SRBC-^{51}Cr (in cpm·10^{-3}), calculated per milligram protein.

Fig. 2. Effect of enhanced antigenic loading on uptake of SRBC-^{51}Cr (a) and salmosan-sensitized SRBC-^{51}Cr (b) by RPM (unshaded columns) and PSPM (shaded columns) populations. a: 1, 2) SRBC-^{51}Cr; 3, 4) SRBC + SRBC-^{51}Cr; b: 1, 4) SRBC-^{51}Cr; 2, 5) SRBC + SRBC-^{51}Cr; 3, 6) SRBC + salmosan-sensitized SRBC-^{51}Cr.

injection of salmosan-treated SRBC-^{51}Cr (Fig. 1b). It can thus be concluded from these results that reduction of uptake of SRBC in the RPM population when injected simultaneously with salmosan is connected with the action of the latter both on the receptor apparatus of RPM and on SRBC. Since salmosan is a polysaccharide, it can be postulated that an important role in the attachment of SRBC to RPM is played by sugar-recognizing lectin-like receptors. The absence of response of PSPM to salmosan indicates that one of the differences between these two macrophage populations is the different state of their surface receptors.

The level of uptake of SRBC-^{51}Cr (3.5 × 108 cells) by RPM and PSPM populations was identical (Fig. 2). The writers showed previously that PSPM obtained 30 min after intraperitoneal injection of SRBC into CBA mice, show a sharp decline in their subsequent capacity for reuptake of SRBC-^{51}Cr in vitro, i.e., PSPM can take up a definite number of SRBC within a definite period of time [4]. The experiments of series II were therefore undertaken in order to compare uptake of SRBC-^{51}Cr in vivo by RPM and PSPM, under conditions of enhanced red cell loading. Mice were given an injection of $3.5 \cdot 10^8$ unlabeled SRBC, followed 1 h later by the same dose of SRBC-^{51}Cr. Peritoneal macrophages were obtained 1 h after the 2nd injection of SRBC and the number of SRBC-^{51}Cr taken up by the RPM and PSPM was determined. The 2nd injection of SRBC-^{51}Cr into the mice led to a sharp increase in their uptake by the RPM population and to a sharp decrease in their uptake by the PSPM population (Fig. 2a). Thus the response of the two peritoneal macrophage populations studied to enhanced loading with the antigen was opposite in direction.

To determine whether the increased uptake of SRBC-^{51}Cr after a 2nd injection of them was connected with the greater abundance of lectin-like receptors in the RPM than the PSPM population, the experiments of series III were carried out. The difference between this series of experiments and the previous series was that the SRBC-^{51}Cr injected twice into the mice had been treated beforehand with salmosan. In the RPM population the effect of increased uptake, observed after repeated injection of SRBC-^{51}Cr, was abolished and uptake fell below the control level, if the SRBC had been treated beforehand with salmosan (Fig. 2b). Treatment of the SRBC-^{51}Cr to be reinjected with salmosan did not give rise to any such effect in the PSPM population.

Experiments carried out with enhanced loading of the macrophages with SRBC showed that the mechanism limiting or enhancing uptake of an increased dose of SRBC by the PSPM or RPM population of CBA mice also is connected, evidently, with differences in the state of the receptor apparatus of these cell populations.

The writers showed previously that injection of SRBC-^{51}Cr into mice simultaneously with salmosan leads to a decrease in their uptake by RPM [2]. In the light of the results it will

be evident that this effect of salmosan is connected with its effect on the membrane both of RPM and of SRBC. The mechanism of adhesion of nonopsonized particles to macrophages is known to involve the sugar regions on the surface of the microbial or other cell [6, 9, 10]. The presence of sugar-specific receptors also has been demonstrated on the SRBC membrane [8, 12]. In the present experiments sensitization of SRBC by salmosan evidently led to screening of these receptors, as a result of which the mechanism of adhesion of SRBC to macrophages may have been disturbed. This was apparent in the series of experiments with enhanced loading of macrophages by SRBC, when enhanced SRBC uptake was followed by a sharp decline. The greater decline in SRBC-^{51}Cr uptake in the case when they were injected simultaneously with salmosan, compared with injection of salmosan-sensitized SRBC, may be evidence of screening of the lectin-like receptors on the RPM themselves also.

When the results are analyzed, the ability of salmosan to influence the phagocytic function of RPM, but not of PSPM, will be noted. In fact, uptake of SRBC by the PSPM population was unchanged either by simultaneous injection of SRBC and salmosan or by injection of salmosan-sensitized SRBC, whatever the dose of SRBC. The differences discovered were evidently connected with differences in the level of expression of lectin-like receptors in these two populations of macrophages, namely their greater expression in the RPM population and their much lower degree of expression in the PSPM population.

Differences in the response of these populations to injection of SRBC were manifested only when loading of the macrophages with them was increased. Stimulation of uptake observed on SRBC loading in the RPM population and the decrease in SRBC uptake in the PSPM population also are evidence of differences in the level of expression of lectin-like receptors in these two macrophage populations. Since lectin-like receptors are essential for adhesion of nonopsonized SRBC, the increase in SRBC uptake by the RPM population under conditions of enhanced loading can be explained by the higher level of expression of these receptors on RPM than on PSPM.

Data obtained previously, showing that in response to injection of SRBC simultaneously with salmosan their uptake by the RPM population decreases, and this is followed by an increase in the number of antibody-forming cells in the spleen, suggest that induction of antibody synthesis does not require a large number of SRBC. Salmosan behaves in this situation as a regulator, improving processing and further presentation of the antigen. The reaching of a definite "threshold" in the quantity of antigen taken up is revealed by comparison of groups 1, 2, and 3 in Fig. 1, where the limit of decline of the level of SRBC-^{51}Cr uptake in cases when 3.5×10^8 SRBC were used and in cases of enhanced RPM loading by SRBC is about equal.

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