Opsonic activity against zymosan of normal human serum of different blood types[1]

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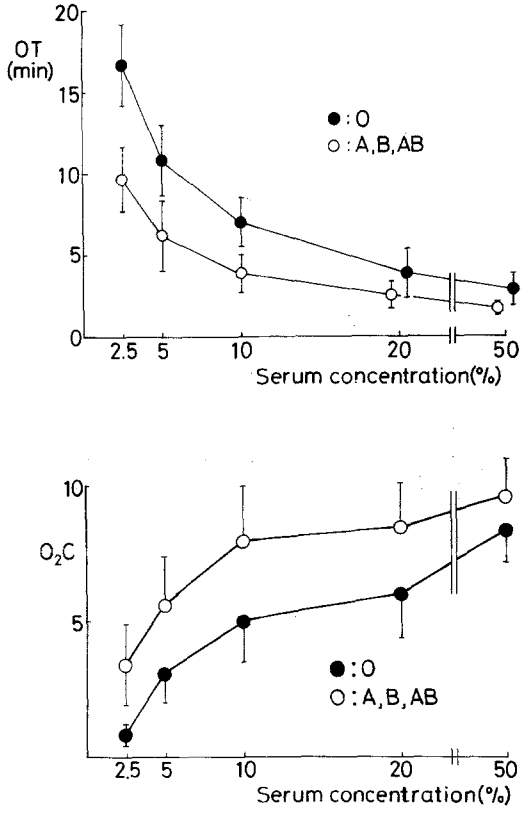
Summary. Serum opsonic activity against zymosan was found to be decreased more markedly in highly diluted O type serum than in the other 3 types, among which no difference was noted. Heat-killed bacteria were equally well opsonized with any type of serum. This peculiar activity of O type serum against zymosan was not affected by the removal of isohemagglutinins. No inhibitory activity was demonstrable in O type serum.

In experiments with combinations of human polymorphonuclear leukocytes and serum, as in phagocytosis or bactericidal assays, cells and serum of the same blood type, O cells with any serum, or AB serum or simply a pooled serum for any type of cells are in general use. When the oxygen consumption capacity of leukocytes was determined with the combination of zymosan and human serum as opsonin, it was found that the opsonic activity against zymosan varied among blood types as well as with the dilution of the serum. The purpose of this brief communication is to present the finding that O type serum becomes less opsonic against zymosan at higher dilutions than other types of serum and to call attention to the importance of this fact when kinetic studies are to be conducted with this combination.

Materials and methods. Blood specimens were obtained from 20 adult volunteers; type A, 8 persons; O, 6; B, 4 and AB, 2, chosen to correspond to the blood type incidence in Japan. Polymorphonuclear leukocytes (PMNL) were isolated from heparinized venous blood following 3% dextran sedimentation, Ficoll-sodium metrizoate gradient and hypotonic lysis of residual erythrocytes, and suspended in HEPES buffer (17 mM N-hydroxyethylpiperazine-N'-ethanesulfonic acid, pH 7.4, 120 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$ and 5 mM glucose) to contain 2×10^6 PMNL. Serum was separated from the specimen without heparin. Zymosan A from *S. cerevisiae* (Sigma Chem. Co.) was washed and suspended in HEPES buffer and used at a concentration of 0.7 mg/10^6 PMNL. Reference strains of *Staphylococcus aureus* 209P, *Escherichia coli* NIHJ-JC2 and *Streptococcus faecalis* were cultured in brain-heart infusion broth for 18 h, washed and heat-killed, and used at a bacteria-to-PMNL ratio of 100. Oxygen consumption during phagocytosis was measured polarographically with a Clark type oxygen electrode (Yellow Springs Instrument Co.) by the method of Nakamura et al.[2]. The total reaction volume was 1.0 ml, which contained PMNL and serum to be examined at specified concentrations. Determination of opsonization time was done by the methods of Nakamura[3] and Markert and Frei[4], which can be briefly summarized as follows. When unopsonized zymosan was added to PMNL suspended in test sera of different concentrations, the oxygen started to be consumed after a certain interval, which depended on the opsonic content. Opsonization time was defined as the interval between the addition of the particle and the point where 2 extrapolated lines intersect, one an extension of the resting phase and the other a linear portion of the phagocytizing phase. The amount of oxygen consumed was expressed as nmoles/10^6 PMNL/min. Serum and PMNL were from the same donor. Removal of isohemagglutinins in type O serum was done with the use of NeutrAB reagent (DADE Division, American Hospital Supply Corporation) and was confirmed by the absence of agglutination of type AB erythrocytes.

Results. The figure shows the oxygen consumption and opsonization time with the different concentrations of serum. As types A, B, and AB sera showed no difference, the results with each type of serum were combined. Although at each concentration, O type serum showed less oxygen consumption and a more prolonged opsonization time, the difference became significant for 2 indices at 10% and lower serum concentrations ($p < 0.01$ at 10 and 5%, $p < 0.001$ at 2.5%, respectively). No difference was noted between type O and the other types of serum when the phagocytosible particles were bacteria. Neutralization of isohemagglutinins in O type serum did not restore opsonin activity. Treatment of the other types of serum with NeutrAB reagent did not affect the results either. The addition of type O serum to other types of serum did not cause inhibition. A highly inverse correlation was noted between oxygen consumption and opsonization time ($r = -0.99$ for type A, B, AB sera and $r = -0.97$ for type O serum). When added to AB serum, O cells showed an oxygen consumption almost equal to that of the other types of cells and the opsonic activity of O serum did not change significantly with any type of cell. Heat-treatment (56 °C, 30 min) of any serum caused loss of opsonin activity. CH50 and C3, C4 and C5 levels were within the normal range with any type of serum.



Opsonization time (OT) and oxygen consumption (O_2C, nmoles $O_2/10^6$ PMNL/min) affected by various concentrations of serum of blood type O (n = 6), A(n = 8), B(n = 4) and AB(n = 2). Results of A, B, and AB are combined. Values are expressed as a mean ± 1 SD.

Discussion. In experiments in which human serum and polymorphonuclear leukocytes are examined for their phagocytic or bactericidal activity, combinations of cells and serum of the same blood type, of O cells with any other type of serum, or of AB serum or pooled serum of nonspecified blood type with cells of any type, are in general use. So far only a few articles have dealt specifically with the problem of the effects of ABO blood types on phagocytosis. With combinations of leukocyte-rich buffy coats and *Staphylococcus aureus,* Tandon[5] examined the phagocytic activity of leukocytes of different blood types and found that O cells have the highest activity, followed by A, B and AB. He speculated that blood group substances in the leukocytes or isohemagglutinins may affect the opsonization of bacteria, although no substantial evidence was offered. When the phagocytic activity of leukocytes was compared in compatible and incompatible sera, Melby[6] noted that O cells are the least and AB cells the most active and that the use of incompatible serum, mostly O serum, generally leads to a reduced uptake of *E. coli.* Therefore, he emphasized that full compatibility is essential in the study of phagocytosis. One of the observations of Melby relevant to the present study is that heat-treatment of serum abolishes the difference, i.e., the serum factors responsible for the reduction of phagocytosis are in the heat-labile fraction. As noted above, heat lability is a feature of opsonin activity against zymosan. Although it remains to be determined whether or not heatstable factors contribute to the opsonization of zymosan, these independent observations indicate that the removal of presumably heat-stable isohemagglutinins does not affect the opsonin activity of O serum. The present experiment shows that a higher dilution of O serum causes a more pronounced decrease in the capacity to opsonize zymosan than dilutions of any other type of serum. This defect appears to reside in the serum, as O cells can consume oxygen equally well when combined with A, B, or AB serum. This decrease of opsonin activity occurred only against zymosan, and contrary to previous reports, bacterial species were not influenced at all by differences in ABO type. The use of live or killed bacteria and their greater response to antibodies as opsonins may explain the difference, but it is not yet completely understood. Such high dilutions of serum are usually not used for opsonization, so the defect revealed in this experiment may not have any practical significance. However, this finding should be considered when kinetic studies of opsonization are conducted with zymosan, and possibly with other particles, in various combinations of sera and cells.

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Effect of azimexon (BM 12.531) on mouse granulocyte-macrophage and monocyte-macrophage progenitor cells[1]

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Summary. Treatment of mice with 25 mg/kg azimexon (BM 12.531) resulted in an increase in granulocyte-macrophage colony-forming cells (GM-CFC) in spleen and bone marrow after a transient depression in the cell populations. Bone marrow monocyte-macrophage colony-forming cells (MM-CFC) increased at 7 days after treatment, and splenic MM-CFC were least affected by azimexon treatment. The increase in granulocytic and monocytic colony-forming cells may play a role in the previously reported protection by azimexon against radiation and drug-induced toxicity.

A series of cyanoaziridine compounds have been studied recently for their immunomodulatory properties[2-7]. Azimexon (BM 12.531, chemical name 2-[2-cyanaziridinyl-(1)]-2-[-2-carbamoylaziridinyl-(1)]-propane) has shown promising results in a number of immunological evaluations. Treatment of mice with azimexon resulted in an increase of delayed-type hypersensitivity reactions[2,3], improved resistance to infections[4,5], activation of macrophages in vivo and in vitro[2,3], augmentation of natural killer cells[6], and prolongation of mean survival time of tumored mice[3]. Addition of azimexon to human lymphocyte cultures in vitro resulted in an enhancement of their blastogenesis in response to mitogens[7], whereas a dose-dependent activation of T lymphocytes from patients treated with azimexon has also been reported[2]. Azimexon treatment increased the survival of mice given cyclophosphamide[8], and the survival of mice receiving total-body X-irradiation was increased when the drug was administered either before[3] of after[8] irradiation. There is evidence that azimexon helps reverse the leukopenia resulting from irradiation[8], cyclophosphamide treatment[4], or combined cytoreductive chemotherapy[9], which suggests a useful adjuvant role of this drug in cancer therapies or in treatment of radiation accident victims.

One of the many aspects of stimulation of the immune system by biological response modifiers is their effects on stem and progenitor cells. The hematopoietic stem cell, CFU-s, is the ultimate precursor of erythrocyte, granulocyte, macrophage, megakaryocyte, and eosinophil differentiated progeny[10]. Two progeny of the CFU-s, the bone marrow MM-CFC[11] and the GM-CFC[12], are of special interest when considering the reticuloendothelial system, since they are early or primitive cells in the sequence from stem cell to mature end cell. The GM-CFC is committed to the granulocyte and mononuclear phagocyte pathway at differentiation, and the MM-CFC is committed exclusively to the mononuclear phagocyte pathway. In considering possible mechanisms for the immunoprotection shown by