Defective Bactericidal Activity of Monocytes in Fatal Granulomatous Disease

By Glenn E. Rodey, B. H. Park, Dorothy B. Windhorst and Robert A. Good

FATAL GRANULOMATOUS DISEASE of childhood (FGD), first defined as a distinct syndrome by Berendes, Bridges and Good in 1957,1-2 represents an immunologic deficiency syndrome in which peripheral white blood cells are unable to efficiently kill certain strains of ingested bacteria. In most of the cases originally described, the syndrome is inherited as a sex-linked or sex-limited disease.3 Several females expressing this syndrome have now been described and an autosomal recessive inheritance has been suggested for some cases.4-7 The killing defect was first demonstrated by Holmes, et al.,5 using an in vitro test which measures the capacity of white cells to ingest and to kill bacteria. Children with this disease characteristically have elevated white counts composed mainly of neutrophils, and the test as it is commonly performed measures primarily the activity of this cell type.

Several clinical features distinguish this disease from other diseases characterized by either quantitative or qualitative defects in neutrophil function. One of the characteristics of FGD is the unexplained granulomatous response to inflammation in the lymph nodes, spleen, liver, skin, lung and kidneys. Additionally, the fixed macrophages in these patients contain inordinate accumulations of so-called lipochrome pigment.8-12

If this functional abnormality were confined to neutrophils, it would be difficult to understand the host-parasite relationships leading to the regular occurrence of granulomatous disease. If, on the other hand, monocytes and other phagocytic cells as well as neutrophils show the functional deficiency, the relation of a generalized phagocytic defect to granulomatous inflammation might be more readily analyzed.9 The intent of this investigation was to

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*That a similar defect in the monocyte does exist is suggested by a recent abstract14 which describes a decreased capacity to reduce nitroblue tetrazolium, by monocytes as well as neutrophils in FGD.
determine whether a killing defect similar to that found in the neutrophils is present in monocytes.

**MATERIALS AND METHODS**

Monocyte suspensions were prepared by modification of a flotation method described by Bennet and Cohin.\(^{13}\) Twenty ml. of venous blood was collected in heparinized plastic syringes and then transferred to sterile plastic centrifuge tubes containing 4 ml. of 6 per cent low molecular weight dextran solution. The cells were allowed to sediment for 45 minutes and the plasma and buffy coat were removed and centrifuged at 800 \(\times\) g. for 12 minutes at 12 C. to remove platelets. The supernatant was removed and a quantity of heparinized 25 per cent human serum albumin (Cutter Laboratories) was added to the cell button to give a final concentration of 20 per cent albumin. This suspension was centrifuged at 2400 \(\times\) g. for 36 minutes at 12 C. Under these conditions, the majority of neutrophils and lymphocytes are spun to the bottom of the tube, whereas most monocytes and some lymphocytes float to the top of the albumin. This thin pellicle is carefully removed with a Pasteur pipette and placed in 10 ml. of 1 per cent gelatin in Hanks balanced salt solution containing 100 units of aqueous heparin. This suspension is centrifuged at 800 \(\times\) g. for 12 minutes at 12 C. and all but 1 ml. of the supernatant is removed. The cell button is thoroughly mixed into the remaining ml. of supernatant and an aliquot is removed for smears, and cell counts, viability studies, including trypan blue exclusion, and ability to phagocytize latex particles indicated that 98 per cent of the cells functioned normally in these parameters. Differential counts by the method of collection averaged 60–80 per cent monocytes, 20–35 per cent lymphocytes and less than 5 per cent neutrophils.

Cell cultures were prepared by a modification of the method described by Holmes.\(^9\) A culture of Staphylococcus aureus was incubated overnight at 37 C. in a shaking water bath, spun at 2000 g., washed twice in isotonic saline and adjusted to a final concentration equivalent to 0.3 O.D. at 650 mm. This cell suspension was then diluted 1/50 with gel-Hanks solution. The culture medium was a 25 per cent solution of pooled normal human serum in Hanks. Each culture contained 0.2 ml. culture medium, 2.5 \(\times\) 10^6 viable monocytes and approximately 1 \(\times\) 10^6 bacteria. The total culture volume was 0.5 ml. 0.01 ml. of penicillin-streptomycin solution to give a final concentration of 100 units penicillin and 100 micrograms of streptomycin was added to each culture approximately 30 minutes after their preparation and the cultures were incubated at 37 C. The cultures were then sampled at 1½ and 3 hours and plated with nutrient agar into sterile 100 \(\times\) 15 mm. petri dishes. Following a 48 hour incubation at 37 C., the number of staphylococcal colonies were counted per plate and the data expressed as the log. 10 number of viable bacteria per ml.

**RESULTS**

In Figure 1 are summarized observations in which neutrophil-rich and monocyte-rich preparations from normal persons are compared with respect to capacity to kill ingested staphylococci. The number of surviving intracellular bacteria in monocyte-rich and neutrophil-rich cell suspensions per individual control is similar. The variation between individuals reflects in part the fact that the tests were run on different days. In these experiments, suspensions of the two cell types were obtained from two blood samples taken from the same donor within 24 hours of one another and run simultaneously.

Figure 2 compares the number of viable bacteria surviving at 1½ and 3 hours following incubation with monocyte-rich cell suspensions from four patients with FGD, and similar preparations from normal controls. Approximately one log. difference in number of surviving bacteria was consistently
present between monocyte preparations of patients and controls at 1½ hours and an even greater difference was obtained at three hours of incubation. This finding suggests that monocytes from patients with FGD kill staphylococci less well than do monocytes from normal persons and that the functional defect is comparable to that previously observed in neutrophil-rich leukocyte preparations.9-11

Prior studies showed that carrier females can be identified by a deficiency of bactericidal action of their neutrophils.3 The neutrophils of carriers, in general, kill staphylococci less efficiently than did normal neutrophils but more efficiently than did neutrophils from their affected sons. In studying neutrophil function of the mothers of affected patients, considerable variation in the degree of functional deficit was observed. It seemed of interest both to determine whether the mothers showed abnormalities of monocyte function and whether the defect of monocyte function if it existed varied in parallel with defects of neutrophil function. For purposes of this analysis the monocyte
Fig. 2.—Each patient is indicated by two letters. The matched controls are preceded by the letter C.

and neutrophil bactericidal activity of a selected group of mothers and patients were compared. Four mothers were chosen, two of which were known to have neutrophils which functioned abnormally in the bactericidal assay. A third mother has shown the defect inconstantly and the fourth has been consistently normal. In Figure 3 are shown comparisons of the killing of staphylococci by monocyte-rich preparations from mothers of affected children and normal controls. The figure reveals considerable variation of the monocyte function in the carriers. Two of the known carrier females studied here showed significantly slower rates of killing of staphylococci than did the control monocytes analyzed simultaneously. In two of four, however, a functional defect of the monocytes of the carrier was not demonstrated.

Since a numerical comparison of monocyte and neutrophil function tests of the carriers is impossible from our data because the tests were done at different times; and under different conditions, we chose to compare the defects of
neutrophil and monocyte function of each mother according to whether the studies of function had revealed in the carrier 1) a functional defect similar to that of the affected patient, 2) a functional defect intermediate between the patient and normal control, or 3) function of cells similar to that of the normal controls. This comparison is presented in Table 1. It will be seen that a similar pattern exists between killing capacity of neutrophils and monocytes in the carrier females, i.e., the monocytes of mothers showed
CORRELATION OF NEUTROPHIL AND MONOCYTE STUDIES OF CARRIERS

Table 1.—Compares Neutrophil and Monocyte Studies Done at Different Times (see text). The number of determinations are shown in parentheses.

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Neutrophil-Rich</th>
<th>Monocyte-Rich</th>
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<tr>
<td>M.K.S.</td>
<td>Interm. (2)</td>
<td>Interm. (1)</td>
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<tr>
<td>M.P.N.</td>
<td>Interm. (2)</td>
<td>Severe (1)</td>
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<tr>
<td>M.D.B.</td>
<td>Normal (3) Interm. (1)</td>
<td>Normal (1)</td>
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<tr>
<td>M.R.C.</td>
<td>Normal (2)</td>
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Discussion

These observations extend to the monocyte a killing defect previously shown to be present in neutrophils of patients with FGD. Neutrophil-poor, monocyte-rich preparations of peripheral blood from these patients failed in every instance to kill staphylococci as effectively as did comparable monocyte preparations from the peripheral blood of normal persons. As with neutrophils, monocytes from the peripheral blood of the mothers of these patients often showed defective killing of staphylococci. However, as with neutrophils, this functional defect is not uniformly expressed or recognized by present testing procedures in the mothers. A similar pattern, however, does exist between the monocyte and neutrophil function in individual mothers. It seems likely that the presence of this defect in different types of phagocytic cells may explain facets of this disease not observed in other diseases having either a quantitative or qualitative defect in neutrophil function. For example, susceptibility to opportunistic invaders, or low-grade pathogens, might be expected from a defect of neutrophil function but granulomatous inflammation would not. Furthermore, patients with agranulocytosis or leukemia lacking mature neutrophils are susceptible to the same class of organisms that plague children with FGD, but such infections tend to be acute and tend to become rapidly disseminated. By contrast, patients with FGD are likely to experience an indolent process featured by granuloma formation and more chronic purulent
phenomena. It seems to us likely that the indolence of infection in FGD could be due to the fact that the defect is generalized in all phagocytic cells but is not absolute. Further, one might speculate that granuloma formation could reflect a relationship of the bacteria and phagocytic cells which is similar to that observed in normal cells confronted with organisms which cannot be properly digested, such as mycobacteria or brucella.

Extension of recent studies with extensively burned patients to include analysis of monocyte function might provide crucial evidence on this point. Alexander et al.\textsuperscript{15} have related increased susceptibility to infection with low-grade pathogens to defective bactericidal function of neutrophils. This, in turn, seems attributable to depletion of lysosomal enzymes. Such patients develop purulent but not granulomatous inflammation. The difference between the two conditions produced by the same organisms in burned patients, patients with agranulocytosis and those in patients with FGD may be due in part to a defect in monocyte function in the FGD patients which is not present in the other conditions.

Since these observations extend the defect of cellular function in FGD to the monocyte, it follows that the function of the fixed macrophages must now be studied in these patients. The existence of a functional abnormality in the fixed macrophage population in patients with FGD, we believe, is signalled by the lipochrome histiocytosis which is such a uniform pathologic feature in these children.\textsuperscript{12} Fixed macrophages may fail to digest normally lipid membranes or perhaps other substances which they engulf and that for this reason they contain abundant lipochrome pigment. Direct analysis of the fixed macrophages in children with FGD is in progress.

**Summary**

The bactericidal activity of monocyte-rich cell suspensions obtained from the peripheral blood of patients with fatal granulomatous disease (FGD), their mothers and normal controls was studied. Defective killing of Staphylococcus aureus, Strain 502A, by monocyte-rich cell suspensions from children with FGD was found when compared with monocyte-rich suspensions from normal controls. This defect, less severe, was also observed in the mothers of affected patients. In this selected population of mothers, the monocyte defect varied in parallel with the previously described neutrophil defect.

**SUMMARIO IN INTERLINGUA**

Esseva studiate le activitate bactericida de suspensiones cellular nc in monocytos obtenite ab le sanguine peripheric de patientes con letal morbo granulomatose (LMG), br matres, e normal subjectos de controlo. Esseva constatate un defective activitate bactericida contra Staphylococcus aureus, linea 502A in suspensiones cellular nc in monocytos ab juveniles con LMG in comparation con suspensiones cellular nc in monocytos ab normal subjectos de controlo. Le mesme defecto—a grados minus sever—eseva observate etiam in le matres del afflicite patientes. In iste population de matres, le defecto monocyctic variava in parallela con le previemente descritibe defecto neutrophilic.

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REFERENCES


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