Defective Opsonization in Multiple Myeloma

By Bruce D. Cheson, Robert R. Plass, and Gerald Rothstein

The mechanisms responsible for the unusual susceptibility of multiple myeloma (MM) patients to infections are incompletely defined. Since MM is associated with decreased production of normal serum proteins, we investigated the possibility that the production of opsonins might also be impaired. The neutrophil chemiluminescence assay of opsonization was used to evaluate the ability of serum from patients with MM to opsonize zymosan. It was found that sera from 18 MM patients exerted only 50% ± 2.5% (mean ± SEM) of the opsonic activity found in 18 control sera (p < 0.001). In mixture experiments, untreated normal serum completely restored the opsonic activity of MM serum, suggesting a deficiency of opsonic factors rather than an inhibitor. In other mixture experiments, heat-inactivated normal serum only partially corrected the opsonic defect in MM serum. Serum from three patients had low C3 levels, and treatment of untreated normal serum completely restored the opsonic activity of MM serum, suggesting a possibility that the production of opsonins might also be impaired. The neutrophil chemiluminescence assay of phagocytosis might also be decreased.

MATERIALS AND METHODS

Patients

Eighteen patients with MM or Waldenström’s macroglobulinemia (WM) were studied. Their treatment history ranged from untreated to several months of combination chemotherapy with a variety of agents. No patient was studied within 3 wk of corticosteroid administration. Two patients with benign gammopathies were also evaluated. Infectious episodes were considered significant if sepsis or pneumonia was documented or if an infection required hospitalization.

Materials

Zymosan A was obtained from Sigma Chemical Co. (St. Louis, Mo.), Hanks’ balanced salt solution (HBSS) from GIBCO (Grand Island, N.Y.), and Macrodex (6% solution of dextran, mol wt 70,000) from Pharmacia Laboratories (Piscataway, N.J.).

Preparation of Granulocytes

Human granulocytes were collected from normal volunteers as described previously.26 After isolation, the cells were suspended in HBSS at a concentration of 10⁷ cells/ml, of which 83%-95% were granulocytes.

Preparation of Serum

Serum was obtained from venous blood from either MM patients or normal controls.

Opsonization

Opsonization was measured by the neutrophil chemiluminescence (CL) assay described by Allen and coworkers27 using the previously described modifications.26 Fifty milligrams of zymosan were incubated with either normal or MM serum, or a mixture of the two, in sufficient HBSS to bring the volume of the suspension to 2 ml. The samples were vortexed and incubated for 30 min at 37°C in a Dubnoff Metabolic Shaker (Lab-line Instruments, Melrose, Ill.). The samples were then spun at 270 g for 10 min in an International HN-S centrifuge (International Products, Needham, Mass.), washed twice in HBSS, and resuspended in HBSS at a concentration of 25 mg zymosan/ml.

CL was assayed using an Iso-cap 300 liquid scintillation counter (Nuclear Chicago, Searle Industries, Des Plaines, Ill.) in the off-coincidence mode with tritium window settings. The constituents of
Fig. 1. Opsonization of zymosan by normal and MM serum. The mean values for groups of 18 sera are shown with the brackets representing the SEM. Values for normal serum were all significantly greater than comparative values for MM serum (p < 0.001).

Fig. 2. Correction of the opsonic defect in MM serum with the addition of normal serum. The mean values for groups of 18 sera are shown with the brackets representing the SEM. The mixture of MM and normal (NL) serum corrected the opsonic defect seen with MM serum alone (p < 0.001 throughout the incubation), although not to a value significantly greater than with NL serum alone.

RESULTS

Opsonization Studies

In initial studies, we treated zymosan particles with various concentrations of normal serum and then exposed this opsonized zymosan (OZ) to normal neutrophils. It was observed that maximal CL occurred if particles were incubated with serum diluted with HBSS at 1:2. For subsequent experiments, we selected a serum concentration of 1:8, because this resulted in CL that was inframaximal but was adequate for measurable light emission. Thus, it was assured that opsonization of both greater and less than control values could be appreciated.

Opsonization of Zymosan With Normal (NL) or MM Serum

Zymosan was opsonized with either normal serum (OZ-NL) or MM serum (OZ-MM) and the CL by normal neutrophils in response to these measured serially over a 40-min period. Using serum from each of the 18 MM patients, MM-OZ stimulated less CL than did NL-OZ. Composite curves of CL, expressed as the mean value for the 18 MM-OZ or 18 NL-OZ, are shown in Fig. 1. It can be seen that at each observation time after 4 min of incubation, the CL in response to MM-OZ was less than with NL-OZ (p < 0.001). Peak CL from MM-OZ was 50% ± 2.5% (mean ± SEM) of that for NL-OZ.

We postulated that the data could have been explained either by the presence of an inhibitor of opsonization or by a deficiency of opsonizing activity in MM serum. In order to explore the possibility of an inhibitor, mixing experiments were performed. The ability of a mixture containing both 1:8 normal serum and 1:8 MM serum to opsonize zymosan was compared with the opsonic ability of either 1:8 normal serum or 1:8 MM serum alone. The results of these studies, shown in Fig. 2, illustrate the correction of the MM opsonization defect by normal serum. No inhibitory effect on opsonization by MM serum was detected.

The next series of studies was designed to evaluate the role of heat-labile opsonins in patients with MM. First, the effect of heat inactivation (56°C for 30 min) was studied. Zymosan opsonized in heated serum from normal or MM subjects did not stimulate CL by neutrophils, consistent with previous reports describing the heat-lability of some opsonic factors. Next, mixtures of 1:8 heat-inactivated normal serum plus 1:8 MM serum were used to opsonize zymosan. This mixture used in particle preparation gave an intermediate CL when compared with the activity of a
A mixture of MM and normal serum (Fig. 3). Nevertheless, a mixture of 1:8 HINS and 1:8 normal serum resulted in opsonic activity no different from 1:8 normal serum alone. These observations suggest that serum from patients with MM may lack both heat-labile and heat-stable opsonic activity. When serum complement concentration was assayed, it was found that the opsonic activity was defective not only in 6 patients with either low C3 or C4, but also in the remaining 12 patients with normal or elevated serum complement. At peak CL, the mean defect in those sera deficient in C3 but not C4, however, was significantly greater than either those with a normal concentration of C3 or the patient population as a whole ($p < 0.02$).

**Clinical Data**

Clinical information for the 18 MM patients is shown in Table 1. Seven patients experienced infectious episodes over an 18-mo period of observation and, in five cases, these were fatal. Two additional patients died at home of unknown causes. The defect noted in the nine infected or expired patients was not different from the patient group as a whole. In the

![Fig. 3. Partial correction of the opsonic defect in MM serum with the addition of heat-inactivated normal serum. The mean values for groups of 18 sera are shown with the brackets representing the SEM. Although the addition of heat-inactivated normal serum (HINS) to MM serum resulted in a significant increment in CL ($p < 0.001$ to 19 min, $p < 0.006$ to 0.01 to 31 min), this was still significantly less than the correction seen with the mixture of MM and NL serum ($p < 0.001$ to peak CL, not significant after 22 min).](#)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>M-type</th>
<th>Spike (g/dl)</th>
<th>C3</th>
<th>C4</th>
<th>Peak CL</th>
<th>Infections</th>
<th>Status</th>
<th>Clinical Information</th>
</tr>
</thead>
</table>
| MA      | 69  | M   | Gκ      | 5.1         | N  | N  | 41     | +          | Dead    | Poor therapeutic response  
Died post pneumococcal sepsis |
| CB      | 77  | F   | Gλ      | 5.0         | N  | N  | 39     | –          | Dead    | Poor response to therapy; amyloid, breast cancer  
Died at home of unknown causes |
| UB      | 47  | F   | Gκ      | 5.3         | N  | N  | 55     | –          | Dead    | Poor therapeutic response  
Amyloidosis |
| EB      | 69  | M   | Gκ      | 3.8         | N  | N  | 64     | +          | Dead    | Transient therapeutic response  
Died septic |
| JB      | 29  | F   | Aκ      | 2.7         | N  | N  | 58     | –          | Alive   | Low-grade astrocytoma  
Stable disease with therapy  
Recurrent pneumonias |
| RE      | 60  | F   | Gκ      | 4.3         | N  | L  | 49     | +          | Alive   | No chemotherapy  
Hyperviscosity, cervical cancer |
| LH      | 65  | F   | Mλ      | 3.0         | N  | N  | 39     | –          | Alive   | Progressive bone disease despite decrease in  
protein spike. H. zoster infection |
| FJ      | 48  | M   | Gκ      | 5.7         | N  | N  | 66     | –          | Alive   | Good response to therapy  
Died septic in renal failure |
| JM      | 52  | F   | Gκ      | 4.0         | N  | N  | 52     | –          | Alive   | Good response to therapy  
Lost to follow-up  
Pneumococcal sepsis as cause of death |
| WX      | 42  | M   | Mκ      | 2.9         | N  | L  | 47     | –          | Alive   | Good response to therapy  
Pneumonia, otitis media |
| JS      | 78  | M   | Mλ      | 3.2         | N  | N  | 59     | –          | Alive   | Good response to therapy  
Hypercalcemia, pneumonia, otitis media |
| DS      | 57  | M   | Aκ      | 4.7         | N  | N  | 57     | +          | Dead    | Poor response to therapy  
Died septic in renal failure |
| LT      | 67  | M   | Aκ      | 4.0         | L  | N  | 27     | –          | Dead    | Multiple bone fractures, severe anemia  
Pneumococcal sepsis as cause of death |
| RV      | 69  | M   | Gκ      | 5.7         | N  | L  | 43     | +          | Alive   | Partial response to therapy  
Died from E. coli sepsis, renal failure,  
hypercalcemia |
| AY      | 66  | M   | Aλ      | 2.1         | L  | N  | 33     | +          | Dead    | Poor therapeutic response  
Died from E. coli sepsis, renal failure,  
hypercalcemia |
| HE      | 60  | M   | k       | –           | N  | H  | 39     | –          | Alive   | Good response to therapy  
Pneumococcal sepsis as cause of death |
| MF      | 84  | F   | Gκ      | 3.1         | N  | L  | 62     | –          | Alive   | No therapy  
Pneumococcal sepsis as cause of death |
| JY      | 53  | M   | λ       | –           | H  | H  | 72     | +          | Dead    | Good response to therapy  
Pneumococcal sepsis as cause of death |
seven subjects with infection, no correlation between residual normal IgG, A, or M and infection could be determined.

No correlation could be demonstrated between the ability of MM sera to prepare zymosan and either the serum paraprotein concentration or the immunoglobulin or light chain subtype. Sera from the two patients with benign gammopathies opsonized normally (mean = 99% peak control CL).

**DISCUSSION**

Bacterial infection is one of the most prevalent causes of death in MM. Up to 84% of patients with MM experience serious infectious episodes, which are fatal in half the cases.\(^1\)\(^2\)\(^5\)\(^6\) Thus, considerable effort has been directed at defining defects in cellular or humoral immunity that might contribute to decreased host resistance in MM. In 1953, Marks\(^9\) observed that patients with MM had decreased serum concentrations of naturally occurring antibodies, and this study was followed by a series of reports describing impaired antibody response to a variety of antigens.\(^1\)\(^3\)\(^21\)\(^22\)\(^29\) In addition, other authors have described increased immunoglobulin turnover,\(^30\)\(^31\) as well as cellular abnormalities of anergy and impaired lymphocyte phytohemagglutinin (PHA) response.\(^32\)

The function of the neutrophil from patients with MM has also been investigated. The majority of patients demonstrate impaired migration of granulocytes into skin windows,\(^11\)\(^12\)\(^25\)\(^32\) yet results with in vitro measures of chemotaxis have not been consistent.\(^25\)\(^33\) Several investigators have evaluated phagocytosis in MM. In 1966, Penny and Galton\(^10\) reported that the addition of MM serum to neutrophils inhibited phagocytic ability as well as adherence to glass beads. MacGregor et al.\(^12\) described inhibition of granulocyte nylon fiber adherence by a factor in MM plasma not present in MM serum. Van Epps and Williams\(^35\) have described that MM-IgA inhibited bacterial killing but did not stimulate neutrophil CL. Therefore, these various observations suggest that a multifactorial defect involving both the humoral and cellular limbs of immunity exists in MM.

In the present study we evaluated the opsonization of zymosan particles by MM serum using the neutrophil CL assay. This assay has been shown to correlate well with measures of specific and nonspecific opsonization,\(^28\)\(^34\)\(^35\) as well as particle ingestion,\(^37\) although the neutrophil is able to generate light with simple membrane interaction with opsonized particles, even in the absence of phagocytosis.\(^38\) Using serum from 18 consecutive patients with MM or WM, we have demonstrated that NL-OZ results in greater opsonic activity than MM-OZ. When we performed mixture experiments to attempt to detect inhibition of opsonization by MM serum, none was noted. Thus, we conclude that MM serum lacks a factor or factors that are necessary for particle opsonization in the CL assay system. It is of interest that, although heat treatment completely eliminated the opsonizing capacity of normal serum, heat-inactivated normal serum still possessed the capacity to enhance the opsonic activity of MM serum. Thus, it can be postulated that heat-stable factors in normal serum interact with factors in MM serum to enhance opsonizing activity. Also of interest is that, although IgG is the most important heat-stable opsonin,\(^15\)\(^16\) we were unable to demonstrate any correlation between the IgG content of MM serum and the degree of the opsonic defect. Heat-inactivated normal serum, however, only partially corrected the defect in MM serum, in contrast to intact normal serum which completely reversed the defect. Therefore, MM serum must also lack heat-labile opsonic activity which, for the most part, is related to the complement system.\(^17\)\(^20\) Nevertheless, the defect was present not only in serum from patients with low C3 or C4 levels but even those with normal or increased concentrations of those complement components. These data suggest that there may be a qualitative abnormality in either the IgG or complement or in the interaction of these two systems in MM serum.

Although one could postulate that defective serum opsonic activity in MM is responsible for the decreased resistance to bacteria exhibited by these patients, we did not observe a clear relation between the defect and patient infections. Thus, it might be speculated that the opsonic defect alone may not be sufficient to impair resistance. However, the hypothesis that the opsonic defect may act additively with other previously described defects to induce susceptibility to infection remains to be evaluated.

**REFERENCES**

5. Twomey JJ: Infections complicating multiple myeloma and
Defective opsonization in multiple myeloma

BD Cheson, RR Plass and G Rothstein

Updated information and services can be found at:
http://www.bloodjournal.org/content/55/4/602.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml