Inhibition of Leukocyte Phagocytic Activity by Hypergammaglobulinemic Multiple Myeloma Plasma.* (26124)

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This report presents preliminary studies on a phenomenon in which the phagocytic activity of peripheral blood leukocytes in an *in vitro* system is virtually completely inhibited by hypergammaglobulinemic multiple myeloma plasma.

Materials and methods. The phagocytic activity of leukocytes is evaluated by means of a procedure used in this laboratory since 1952(1). Briefly, heparinized blood is incubated with saccharated iron† in a rotating plastic vial immersed in a water bath maintained at 37.5°C. After incubation, coverglass blood films are pulled. Saccharated iron in the leukocytes is demonstrated by means of the Prussian blue reaction and the preparations are counterstained with basic fuchsin. For each sample, a minimum of 100 potentially phagocytic cells, *i.e.*, granulocytes and monocytes, are classified individually as being "marked" (containing one or more particles of iron) or "unmarked" (containing no iron).

The ratio of volume of blood to volume of iron must be constant among the incubation mixtures(1). In the present studies this ratio is 100 volumes of blood plus one volume of iron, total volume of each sample being 2-5 ml.

If samples are aspirated from such an incubating system serially over a period of about one hour, the number of potentially phagocytic leukocytes which have failed to ingest iron is found to decrease exponentially with time. Thus, number of "marked" or "unmarked" phagocytic leukocytes per 100 potentially phagocytic cells examined may be used as an index of rate of phagocytosis.

This series of studies represents 13 patients with multiple myeloma (MM), 6 of them with hyperproteinemia due to abnormally high levels of gamma globulin. Studies on 4 patients with hypergammaglobulinemia due to disease other than M.M. are also included. Plasma protein studies were carried out in the Special Determinations Laboratory, Univ. of Rochester Med. Center. Total protein determinations were made by means of the biuret procedure and serum protein fractionation by paper strip electrophoresis.

The basic protocol utilized 4 cell-plasma-iron incubation mixtures. The patient's cells were incubated in (1) patient's plasma and (2) normal compatible plasma; cells from normal compatible blood were incubated in (3) their native plasma and (4) patient's plasma. In some instances a fifth sample was included, consisting of the patient's cells and plasma plus commercially available human gamma globulin containing 16.5 g % γ globulin (Immune Serum Globulin).‡ Cells incubated in plasma other than their native plasma were first washed once with a small volume of the plasma in which they were to be incubated.

The effect of diluting patients' plasma with

* Dr. Scott N. Swisher and associates, Dept. of Medicine, Univ. of Rochester School of Med. and Dentistry, and Dr. Richard F. Platzer, Clifton Springs Sanatorium, Clifton Springs, N. Y. were responsible for diagnosis and care of the patients represented in this report, and called them to the author's attention. One ambulatory patient was under the care of Dr. C. B. F. Gibbs who kindly enlisted the patient's cooperation in the study. The author is grateful to these colleagues for their valuable assistance. Dr. John H. Vaughn and Dr. Vincent Butler have given invaluable consultation on this research. This report is based on work performed under contract with U. S. Atomic Energy Comm. at Univ. of Rochester School of Med. and Dentistry Atomic Energy Project, Rochester, N. Y.

† The commercial preparation, *Proferrin*, kindly made available by Sharpe and Dohm, was used in all the studies.

‡ This material was kindly made available to Medical Division, Univ. of Rochester Atomic Energy Project, by Lederle Labs.

TABLE I. *In Vitro* Phagocytosis by Granulocytes.

Patient	Serum proteins (g %) Total protein	Total globulin	Total γ glob.	% granulocytes ingesting Fe: Pt. cells + Pt. pl.*	Pt. cells + D. pl.	D. cells + Pt. pl.	D. cells + D. pl.
M.M. with hypergammaglobulinemia							
O.R.	10.9	—	6.0	0	—	—	—
P.P.	13	11.5	10.7	0	99	0	51
M.L.	9.7	7.8	6.8	1	100	1	99
N.F.	10.6	8.5	6.4	2.5	90	3.5	98.5
F.S.	12.0	9.3	7.5	0	100	1	87
B.A.	11.2	9.3	8.0	1	83	0	80
M.M. with increased α or β globulins							
N.W.	9.4	6.1	.4	84	98	90	88
P.C.	10.9	8.7	<.1	90	100	—	—
M.S.	9.7†	6.7	.3	80	94	46	79
S.W.	8.1†	5.2	1.0	100	100	100	99
M.M. without hyperproteinemia							
I.O.	7.0	2.1	.4	93	93	89	87
G.B.	6.3	2.6	.7	97	99	97	96
E.W.	6.8	2.9	.9	64	76	98	96
Absolutely or relatively increased γ globulin; not M.M.							
M.M.[1]	7.7	4.2	2.4	100	95	91	98
J.R.[2]	6.1	4.3	1.5	91	98	93	75
G.M.[3]	5.6	3.3	1.6	65	100	86	100
K.B.[4]	9.4	7.0	5.7	78	87	94	98

* Pt. = patient's; pl. = plasma; D. = normal compatible donor's.
† Protein determinations on plasma instead of serum.
[1] Sjogren's syndrome; [2] chronic alcoholism and CNS atrophy; [3] lymphoma; [4] chronic liver disease.

(1) 0.85% NaCl solution and (2) normal compatible plasma was studied in experiments representing 3 myeloma patients with hypergammaglobulinemia.

The possibility that multiple myeloma plasma might simply interfere with the Prussian blue reaction was evaluated as follows: Cells which had been incubated with iron in M.M. plasma were washed twice with normal plasma and suspended in normal plasma before smears were pulled. No intracellular iron was demonstrable in these cells. The counterpart of this experiment was also carried out, *i.e.*, cells which had been incubated with iron in normal plasma were washed twice with multiple myeloma plasma and suspended in it before smears were pulled. The intracellular iron in these cells was demonstrated just as vividly as in cells which remained in normal plasma.

Results. Data are summarized in Tables I, II, III and IV. The salient points are as follows: (1) Hypergammaglobulinemic multiple myeloma plasma inhibits, almost completely, *in vitro* ingestion of saccharated iron by peripheral blood phagocytes from both healthy individuals and M.M. patients (Table I). (2) Plasma of M.M. patients with hyperproteinemia due to increased amounts of α or β globulins rather than γ globulins does not inhibit ingestion of saccharated iron by peripheral blood phagocytes, nor is phagocytosis inhibited by plasma of multiple mye-

TABLE II. Effect of Diluting M.M. Plasma with Saline or Normal Plasma. Patient, F.S. Total protein, 12 g %. γ globulin, 7.5 g %.

	% patient's plasma	% diluent	% marked phagocytes*
Saline diluent	100	0	8
	80	20	13
	40	60	83
	20	80	78
	10	90	52
	5	95	33
Normal plasma diluent	100	0	3
	80	20	5
	40	60	68
	20	80	80
	10	90	81
	5	95	86
	0	100	91

* Cells containing 1 particle or more of saccharated iron.

TABLE III. Effect of Adding Immune Serum Globulin (ISG) to Patients' Cells and Plasma and Saccharated Iron *In Vitro.*

Patient	Serum proteins (g %) Total	Globulins	γ glob. only	% marked phagocytes in: Plasma only	Plasma + ISG	g % γ glob. after adding ISG (calculated)
M.M.* with increased γ globulin						
F.S.	12	9.3	7.5	0	1	9.4
N.F.	10.6	8.5	6.4	2.5	6.5	8.7
B.A.	11.2	9.3	7	1	2	8.9
M.M. with increased α or β globulin						
N.W.	9.4	6.1	0.4	85	87	2.6
M.S.	9.7	6.7	0.3	80	9	2.4
M.M. without hyperproteinemia						
I.O.	6.3	2.6	0.7	89	3.5	2.1
G.B.	6.8	2.9	0.9	99	26	2.2
E.W.	7.0	2.1	0.4	93	7	3.6
Absolutely or relatively increased γ globulin; disease not M.M.						
M.M.	7.5	4.0	2.4	95	88	5.4
J.R.	6.1	4.3	1.5	91	20	4.2
G.M.	5.6	3.3	1.6	65	23	4.4

* Multiple myeloma.

loma patients with normal total plasma protein levels (Table I). (3) Hypergammaglobulinemic M.M. plasma diluted with normal plasma or saline is entirely capable of supporting brisk phagocytosis. Results representing the two types of dilution studies may be compared in Table II. The percentage of phagocytes ingesting saccharated iron increases progressively as the proportion of normal plasma increases, reaching a maximum in 100% normal plasma. Diluting the abnormal plasma with saline also increases phagocytosis. In saline dilutions, however, a minimum of about 20% patient's plasma appears to be necessary for optimum phagocytic activity. Although results of plasma dilution studies for 3 hypergammaglobulinemic M.M. plasmas studied in this way are similar, further studies must be completed to determine how consistent the shapes of the dilution curves may be from one plasma specimen to another. (4) Addition of commercially available immune serum globulin to the plasma of multiple myeloma patients with hypergammaglobulinemia fails to promote ingestion of saccharated iron by granulocytes (Table III). Furthermore, phagocytosis is depressed by addition of normal gamma globulin to multiple myeloma plasma which is not hyperproteinemic or in which hyperproteinemia is due to increased amounts of alpha or beta globulins rather than gamma globulins. Addition of normal γ globulin to normal plasma inhibits ingestion of saccharated iron by phagocytic leukocytes only in concentrations greater than about 5 g% (Table IV). This is in distinct contrast to the concentrations of only 2 to 3 g% required to inhibit phagocytosis in M.M. patients without hyperproteinemia. (5) Incubating particles with commercial immune serum globulin before exposing them to blood cells *in vitro* did

TABLE IV. Effect of Adding Immune Serum Globulin (ISG) to Normal Blood and Saccharated Iron *In Vitro.*

Normal donor No.	% marked phagocytes: Plasma only	Plasma + ISG	γ glob. after adding ISG*
1	88	100	3
2	89	96	3
3	100	99	3
4	95	88	1.2
	95	85	1.6
	95	32	4.5
5	99	60	3
	99	64	3.4
	99	43	4
	99	10	5

* Protein fractionation studies were not carried out on earlier normal plasma samples. These are assumed to contain approximately 1 g % γ globulin. Normal range in Special Determinations Laboratory is 0.6-1.7 g %.

not inhibit phagocytosis in the few instances in which it was tested.

Discussion and conclusions. This report is intended to describe a new observation. Speculation about the ultimate significance of virtually complete inhibition of leukocytic phagocytosis in hypergammaglobulinemic multiple myeloma (M.M.) plasma *in vitro* is clearly unwarranted at present, but a few tentative conclusions are justified: (1) Depressed phagocytic activity is directly attributable to the γ globulin fraction in M.M. plasma and not to a cellular defect. (2) Plasma from M.M. patients with hyperproteinemia due to increased levels of α or β globulins, or from patients with hypergammaglobulinemia due to disease other than M.M. does not inhibit leukocyte phagocytic activity. (3) Inhibited phagocytosis appears not to be solely a function of total gamma globulin level. This is most clearly demonstrated by the relatively low levels of γ globulin which inhibit phagocytosis in plasma of M.M. patients without hypergammaglobulinemia or hyperproteinemia; most notably when γ globulin levels are lower than normal. (4) Since the inhibitory effect of hypergammaglobulinemic M.M. plasma may be decreased or abolished by diluting the abnormal plasma with normal plasma or isotonic saline, the effect is not due to a simple deficiency. Specifically, the inhibitory phenomenon appears not to be due to a deficiency of normal gamma globulin. (5) Studies of the phenomenon are continuing.

1. Ingram, M., Struthers, A., Platt, D., Welton, J., Yettewich, G., *Further Studies on a Procedure for Determining Phagocytic Activity of Leukocytes in Whole Blood by Incubation with Saccharated Iron*, UR-277, Univ. of Rochester Report (1953).

Received Sept. 19, 1960. P.S.E.B.M., 1960, v105.

Some Characteristics of Para-Mucus Secretion.* (26125)

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It has been reported from this laboratory that the topical application of iodoacetamide (IAA) or N-ethyl maleimide (NEM) solutions to the mucosa of canine gastric pouches will evoke a flow of mucinous secretion for many hours(1). This material had never been recognized before, and because its physiological significance is still unknown, it was designated "para-mucus"(2). Exploratory experiments on the pepsin content of para-mucus indicated that the zymogen is present in appreciable quantities, and that it is probably a significant component in chemical characterization of this mucinous secretion. This report presents a systematic study of the variations in pepsinogen content of para-mucus, using specimens collected fractionally every 30 minutes for 6 hours following topical application of IAA solution to the gastric mucosa in dogs with a Heidenhain pouch and antrectomy. Several other characteristics of this material are presented here.

Material and method. Four Heidenhain pouch dogs, provided with an antrectomy, were used in these experiments. The antral resection was performed to insure the virtually complete absence of HCl secretion in the fasting state, in accordance with previous experience in this laboratory(3). Each dog was subjected to the following 2 types of experiment: A) *Histamine stimulation*: Dogs were starved for at least 18 hours before experiment, and unstimulated (basal) secretion was collected for 2 hours, at 60-minute intervals. Following this, histamine was administered subcutaneously (0.1 mg of histamine

* This work was supported by grant from Nat. Cancer Inst., U.S.P.H.S.

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