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Monocyte Metabolic Activation in Patients with Rheumatoid Arthritis (40541)

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The "activated" monocyte (MNP) is distinguishable from the resting monocyte by altered functions (1, 2). These changes include: enhanced phagocytosis, cytoplasmic spreading and microbicidal activity, increased cytoplasmic lysosomal content, enhanced secretion, greater metabolic activity, and stimulated Fc receptor expression (2, 3). Recent evidence indicates that incubation with immune complexes *in vitro* may induce monocyte activation (2, 4). Monocytes and macrophages have the capacity to bind immune complexes via Fc receptors (3) and mouse peritoneal macrophages cultured in the presence of immune complexes secrete lysosomal hydrolases at an accelerated rate (5). It was of interest to determine whether monocytes from patients with rheumatoid arthritis, a disease frequently associated with circulating immune complexes (6, 7), is associated with alteration in monocyte function. We investigated metabolic activity, hexose monophosphate shunt activity (HMP) for monocytes of patients with rheumatoid arthritis (RA) and have also studied the effect of rheumatoid sera on HMP shunt activity of normal monocytes.

Materials and methods. The patients studied were diagnosed as rheumatoid arthritis by the ARA criteria. Mononuclear cells from controls and patients were isolated from venous blood specimens by Ficoll–Hypaque gradient centrifugation (8). The cells were then resuspended in TC 199 with 15% FCS (fetal calf serum) and allowed to adhere to the bottom of 25-ml glass flasks over a 1-hr incubation period at 37° in a 5% CO_2 atmosphere. The nonadherent cells were removed by washing the flasks with TC 199. This adherence procedure routinely results in 90–95% MNPs as determined by latex ingestion and nonspecific esterase staining.

Determination of resting HMP activity for isolated MNPs from control and patients were performed as described previously (9, 10). Briefly, this involves the addition of 2 ml of TC 199 over the MNP monolayer adherent to the bottom of the glass flask, and 100 λ of glucose radiolabeled at the Cl position. Methylene blue, an electron acceptor dye (11) was added to some flasks in order to assay the ability of the HMP shunt to be stimulated (stimulated HMPs). The flasks with a center well in place (from Kontes) are then tightly stoppered and a 2-hr incubation at 37° in a shaking water bath was carried out. Then, 0.3 ml of methyl benzonium hydroxide (from Sigma) was added to the center well and an additional hour of incubation at 37° in a shaking water bath was performed, in order to permit absorption of evolved CO_2 into the reagent in the center well. The center well was then removed, placed in scintillation fluid, and counted in a Beckman beta-scintillation counter. Control MNP were always run simultaneously in this assay for the various experimental protocols used.

The numbers of adherent MNP in the flasks were determined by a spectrofluorometric method utilizing ethidium bromide, an agent that intercalates with DNA (12). This assay assessed cellular micrograms of DNA in the flasks. All counts observed for "resting" or "stimulated" HMPs were then corrected to micrograms DNA.

To evaluate the effect of normal and rheumatoid sera on isolated control MNPs, the adherent MNP monolayers were incubated with 1 ml of the various sera for 1 hr at 37° in a 5% CO_2 atmosphere. The sera were then removed and the MNP monolayers washed with TC 199 before the resting and/or stimulated HMPs assay was performed. Sera were fractionated into 7 and 19 S components by Sephadex G-200 chromatography. The fractions were measured by optical density and then adjusted to equivalent concentrations in TC 199 before incubation with control MNP monolayers. The fractions were then removed and the monolayers washed twice before the HMPs activity was determined.

Results. The effect of rheumatoid sera on

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normal monocytes is shown in Fig. 1. All five rheumatoid sera produced elevations in both "resting" and "stimulated" HMP activity as compared to MNPs incubated with control sera. While the effect varied in degree, there was only one patient's serum that did not result in greater than 10% enhancement of HMP activity. For this small group of patients there was no apparent correlation between this effect, clinical status, rheumatoid factor titer, or therapeutic status. Table I summarizes the rheumatoid sera effects on HMP shunt activity against that of control sera. The mean values of resting and stimulated monocyte HMP activity were significantly higher when exposed to rheumatoid sera.

To more closely detail the components present in rheumatoid sera that are responsible for monocyte HMP activity, we fractionated the rheumatoid sera (N 1–4, Fig. 1) into 7 and 19 S components via Sephadex G-200. Table II shows the levels of "stimulated" HMP activity following incubation of 7 or 19 S fractions with control monocyte monolayers. Only the 19 S fraction of serum number 3 had an enhancing effect. This fraction also had the highest rheumatoid factor titration. None of the 7 S components were effective in raising HMP levels. Similar results were noted for resting HMP values. The 7 and 19 S fractions of each serum were then recombined and again incubated with control monocyte monolayers. Three of the four sera were now able to enhance the stimulated HMP levels (Table III). In addition, these three sera had detectable rheumatoid factor titers, but the nonenhancing serum did not.

To determine whether monocytes from patients with RA have abnormal HMP activity, as compared to monocytes from healthy subjects, monocyte monolayers were prepared from four patients with RA and "resting" and "stimulated" HMP activity determined (Table IV).

The mean "resting" and "stimulated" HMP activity was greater than for control monocytes. The data demonstrates that the

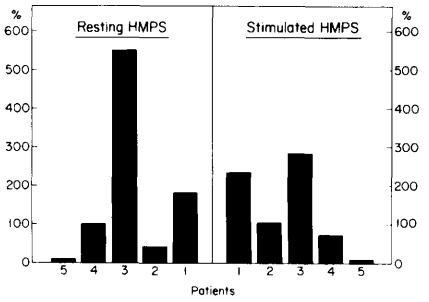

FIG. 1. The effect of individual rheumatoid sera on control monocytes resting and stimulated HMPs activity. The results are expressed as percentage of control monocyte HMPs activity (resting and stimulated) after exposure to rheumatoid sera.

TABLE I. HEXOSE MONOPHOSPHATE SHUNT ACTIVITY[a]

	cpm ± SEM/μg DNA		
	Control sera (n = 5)	Rheumatoid sera (n = 5)	P value
Resting	224 ± 71	368 ± 133	<0.01
Stimulated (methylene blue)	705 ± 244	1296 ± 571	<0.01

[a] The mean values (cpm ± SEM/μg DNA) of control monocyte resting and stimulated HMPs activity following incubation with control (n = 5) or rheumatoid sera (n = 5).

TABLE II. HMPS ACTIVITY (STIMULATED) OF CONTROL MONOCYTES FOLLOWING INCUBATION WITH 7 OR 19 S FRACTIONS FROM RHEUMATOID SERA[a]

	cpm ± SEM/μg DNA	Rheumatoid factor titer
Control	597.5 ± 41.0	
Rheumatoid sera		
1. (a) 7 S	556 ± 27.5	Negative
(b) 19 S	536 ± 36.5	320
2. (a) 7 S	545 ± 27.0	Negative
(b) 19 S	530 ± 28.0	160
3. (a) 7 S	538 ± 23.0	Negative
(b) 19 S	653 ± 26.0	2560
4. (a) 7 S	510 ± 53.4	Negative
(b) 19 S	532 ± 27.9	40

[a] The mean values (cpm ± SEM/μg DNA) of stimulated HMPs activity of control monocytes following incubation with 7 or 19 S fractions from rheumatoid sera.

TABLE III. EFFECT OF RECOMBINATION OF 7 AND 19 S RHEUMATOID SERUM FRACTIONS ON HMPS (STIMULATED) ACTIVITY[a]

	cpm ± SEM/μg DNA	Rheumatoid factor
Control	887 ± 28.0	
Rheumatoid sera		
1	2960 ± 112.0	+
2	854 ± 48.0	0
3	1075 ± 50.0	+
4	3892 ± 150.0	+

[a] The mean values (cpm ± SEM/μg DNA) of stimulated HMPs activity of control monocytes following incubation with the recombined 7 and 19 S fractions of individual rheumatoid sera.

TABLE IV. HMPs Activity of Monocytes Isolated from Rheumatoid Arthritis Patients[a]

	HMPs activity (mean ± SEM/μg DNA)			
	Resting		Stimulated	
Patients ($n = 4$)	180 ± 21	$P < 0.01$[b]	810 ± 110	$P < 0.001$[b]
Controls ($n = 4$)	140 ± 15		480 ± 56	
Individual	Percentage of control		Percentage of control	
1	−5.0		+18.0	
2	−4.0		+34.5	
3	+8.2		+30.2	
4	+10.1		+41.0	

[a] Top half of table: The mean values (cpm ± SEM/μg DNA) for resting and stimulated HMPs activity of isolated monocytes from patients with rheumatoid arthritis in comparison to that of controls. Bottom half of table: Percentage difference of resting and stimulated HMPs activity from control monocytes for each monocyte population isolated from a patient with rheumatoid arthritis.

[b] Student's t test analysis of difference between patients and controls.

stimulated HMP levels were above normal values for all four patients and resting HMP levels were slightly higher for two of the four patients.

Discussion. These findings indicate that rheumatoid sera are capable of enhancing monocyte hexose monophosphate shunt activity. In addition, circulating blood monocytes of patients with RA have higher HMP shunt activity than monocytes from healthy volunteers.

The component in rheumatoid sera that is responsible for modulating this aspect of monocyte metabolism is unclear. However, when the sera were fractionated, activity was lost and could then be recovered by recombining the fractions. This finding suggests that an immune complex was required in order to augment monocyte shunt activity. The immune complexes involved which interact with monocytes might be rheumatoid factor. This is suggested by the fact that three of four reconstituted sera augmented HMP activity, with the sole exception being rheumatoid factor negative. There are, however, many other possible immune complexes which circulate in these patients and may also be involved in potentiating metabolic activity.

It is apparent that some rheumatoid patients have both the capacity to "activate" monocytes, and also have circulating peripheral blood monocytes with enhanced HMP shunt activity. "Activated" monocytes have been demonstrated in other disease states, including sarcoidosis (13), granulomatous bowel disease (14), and immune hemolytic anemias (15). Only in the latter disease state is it reasonably clear what relevance these activated monocytes might have to the pathophysiology of the disease process. However, the monocyte is a mobile cell with an extensive array of secretory substances, including proteases, lysozyme, collagenase, and elastase. It has phagocytic capabilities of both immune and nonimmune nature (3, 16, 17). Therefore, it is uniquely capable of interacting with its environment in either a beneficial or destructive fashion. The monocyte is a characteristic cell type in chronic inflammatory reactions (18, 19), including rheumatoid synovium, and presumably following activation these cells may play a vital role in the disease. It is not evident whether this perturbed metabolism in the monocyte of a patient with rheumatoid arthritis has any relation to the disease process.

The mechanism of producing the altered monocyte metabolism may originate at the cell membrane level (20). This has been shown for PMN leukocytes when oxidative metabolism stimulation may occur with perturbation of cell membrane independent of phagocytosis. Monocyte membrane may also be the originating site of metabolic stimulation in that both detergents and phospholipase may trigger oxidative metabolism (21, 22). The occurrence of a soluble circulating component in rheumatoid sera provides a unique opportunity to further analyze the ability of molecular cell surface interactions in altering cytoplasmic metabolic events.

Summary. Monocytes isolated from pa-

tients with active rheumatoid arthritis had elevated hexose monophosphate shunt (HMP) levels. Metabolic activity of blood monocytes incubated with rheumatoid sera was assessed by measuring hexose monophosphate shunt (HMP) levels. Sera from patients with rheumatoid arthritis enhanced HMP shunt activity of normal monocytes. These findings suggest that blood monocytes may be activated by circulating components present in rheumatoid sera.

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