Vol. 130, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1985 Pages 30-36

STUDIES ON MONOKINES AS MEDIATORS OF THE ACUTE PHASE RESPONSE: EFFECTS ON SIALYLTRANSFERASE, ALBUMIN, α_1-ACID GLYCOPROTEIN AND β-N-ACETYLHEXOSAMINIDASE

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Received May 9, 1985

Rat peritoneal leukocytes (PEC) were fractionated on Percoll gradients to prepare populations of monocytes/lymphocytes and polymorphonuclear leukocytes; adherent (monocyte enriched) and non-adherent (lymphocytes/polymorphonuclear leukocytes) cells were also isolated from PEC. Cytokines were prepared from PEC and subfractions and injected into rats to induce the acute phase reactants serum α_1-acid glycoprotein and sialyltransferase; negative acute phase parameters serum albumin and liver hexosaminidase were also assayed. Monocyte derived cytokines (monokines) mimicked the acute phase response of all four parameters in vivo. The sialyltransferase isoenzyme that responded to monokine was identified as the Galβ1→4GlcNAcα2→6 isoenzyme.

Tissue injury caused by inflammatory agents, infections, and pathological conditions results in biochemical and physiological changes known as the acute phase response (1-4). A major change that occurs is an increase in serum glycoproteins known as the acute phase reactants (5); other proteins like albumin decline and are referred to as negative acute phase reactants (4). Recently we have shown that in rats, hepatic and serum levels of Galβ1→4 GlcNAcα2→6 sialyltransferase are elevated in inflammation (6), hepatic levels of β-galactosidase and β-N-acetylhexosaminidase are depressed (4).

In a previous report (7) we showed that a cytokine from peritoneal exudate leukocytes (PEC) mimicked the acute phase response of the above parameters when given as a single injection to rats. However, the cell type producing the active factors and the substrate specificity of the sialyltransferase were not identified. In this study PEC have been fractionated and a preparation rich in monocytes has been identified as the

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0006-291X/85 $1.50

main source of cytokine that produces the changes in sialyltransferase, hexosaminidase, albumin and α_1-acid glycoprotein that were reported earlier (7-9). Also, we have confirmed that Galβ1→4 GlcNAcα2→6sialyltransferase is the isoenzyme that responds to monokine.

MATERIALS AND METHODS

Rat PEC were prepared as previously described (7). PEC preparations were fractionated on a Percoll gradient (Sigma Chemical Co., St. Louis, MO) or into adherent and non-adherent cell populations. The procedure of Watt et al. (10) was used for fractionation on a Percoll gradient. Washed PEC (7) were suspended at 2 - 5 x 10^7 cells/ml in 20 mM sodium phosphate buffer, pH 7.3, 0.149 M NaCl and mixed with 8 vol isotonic Percoll prepared by diluting 9 vol Percoll with 1 vol 0.2 M sodium phosphate buffer, pH 7.3, 1.49 M NaCl. The mixture was centrifuged at 60000 g_{av} for 20 min in a Beckman L5-50 ultracentrifuge fitted with a Ti60 rotor. Monocytes and lymphocytes formed a band about 1/3 down the gradient (band 1) and polymorphonuclear leukocytes banded about 2/3 down the gradient (band 2). The two bands were collected by aspiration and were sedimented by centrifugation at 2000 g_{av} for 20 min in a Sorval RC-2B. For fractionation into adherent and non adherent cell populations, the procedure described by Optiz et al. (11) was used. This involved plating PEC on Lux plastic dishes at 10^8 cells/ml in 0.15 M NaCl and incubating for 2 hr at 37° in a humid environment under 95:5 air:CO_2. Non adherent cells were aspirated and adherent cells were washed with 0.15 M NaCl at 37° and then dislodged with a rubber policeman. As a control, PEC were incubated as above and the entire cell population was collected. Cytokines were prepared from the two fractions from the Percoll fractionation and from adherent, non-adherent and control PEC as before (7). Leukocyte subpopulations were identified following staining with Wrights stain as described by Humanson (12) by bright field microscopy with a series 1820 Biostar Microscope (American Optical, Buffalo, N.Y.). Measurement of albumin, α_1-acid glycoprotein, sialyltransferase and β-N-acetylhexosaminidase as well as the procedure for pronase treatment of cytokine and administration of cytokine to rats were as previously described (6-7). Sialyltransferase isoenzymes in liver and serum were determined by formation of 6'(α2→6) and 3'(α2→3) isomers of sialyllactose as previously described (6). The isoenzyme that responded to cytokine was the Galβ1→4 GlcNAcα2→6 sialyltransferase; this was confirmed using antiserum raised against rat hepatic α2→6 sialyltransferase. When antiserum was used to characterize the enzyme up to 1 µl were added to samples containing 1 mg liver protein and up to 0.5 µl were added to serum in the standard assay. Antiserum was a gift from Dr. J. Paulson, U.C.L.A.

RESULTS

α_1-Acid glycoprotein, sialyltransferase and albumin were determined at 36 hr after cytokine and liver N-acetylhexosaminidase was at 24 hr after cytokine, the times after cytokine where maximum responses were observed (7). Varying the dose of cytokine from PEC populations showed that all acute phase parameters responded to 50 MCE or greater of cytokine except for N-acetylhexosaminidase which responded to 100 MCE or greater of cytokine (MCE is the

Table 1. Effect on Acute Phase Parameters of Cytokines from PEC Fractionated on Percoll*

Parameter	Response to cytokine; % of controls		
	PEC	Band 1	Band 2
Serum			
α_1-acid glycoprotein	153 ± 6	161 ± 8	104 ± 5
albumin	81 ± 3	83 ± 2	94 ± 3
sialyltransferase	297 ± 24	225 ± 9	116 ± 8
Liver			
sialyltransferase	149 ± 5	129 ± 5	94 ± 4
hexosamindase	88 ± 2	77 ± 4	98 ± 3

*band 1 contained 57% lymphocytes, 14% monocyte/macrophages, 28 % polymorphonuclear leukocytes and 1% mast cells; band 2 contained 98% polymorphonuclear leukocytes, 1% lymphocytes and a trace of monocytes and mast cells. Animals received 180 MCE cytokine i.p. Results are the means ± S.D. of 3 analyses expressed as percentages of pronase controls (7).

cytokine from 10^6 cells (13)); doses up to 500 MCE did not significantly affect the magnitude of the responses. Following fractionation of PEC populations on a Percoll gradient only cytokine from the monocyte/lymphocyte band altered acute phase parameters when given as a single injection (Table 1). Table 2 shows the results obtained with cytokines from adherent and non adherent cell populations. All acute phase parameters responded to the cytokine from the adherent cell population; with sialyltransferase the response was significantly greater than that observed with the cytokine from the

Table 2. Effect on Acute Phase Parameters of Cytokines from Adherent and Non-adherent Cells from PEC Preparations*

Parameter	Response to cytokine; % of controls		
	PEC	Adherent cells	Nonadherent cells
Serum			
α_1-acid glycoprotein	141 ± 7	168 ± 9	109 ± 7
albumin	85 ± 2	83 ± 3	94 ± 4
sialyltransferase	195 ± 2	275 ± 12	121 ± 17
Liver			
sialyltransferase	138 ± 8	152 ± 9	107 ± 6
hexosamindase	57 ± 12	70 ± 9	85 ± 8

*Adherent cells contained 90% monocytes, 8% polymorphonuclear leukocytes and 2% lymphocytes; non-adherent cells contained 80% polymorphonuclear leukocytes, 15% lymphocytes, 4% monocytes and 1% mast cells. Animals received 120 MCE cytokine i.p. see Table 1 for other information.

Table 3. Effect of Three Injections of Monokine from Adherent Cell Populations on Serum α_1-acid Glycoprotein and Sialyltransferase*

Parameter	Response to cytokine; % of controls	
	turpentine (36 hr)	monokine
serum α_1-acid glycoprotein	570 ± 50	260 ± 40
serum sialyltransferase	525 ± 45	370 ± 26

*Monokine was prepared from adherent cell populations from PEC and 100 MCE were given i.p. at zero time then at 12 hr and again at 24 hrs; animals were sacrificed at 36 hr from zero time. See Table 1 for other information.

corresponding control PEC; there was also a response of N-acetylhexosaminidase to the cytokine prepared from the non-adherent cell population.

In the previous studies (7) and those described above the response of acute phase parameters to cytokine was lower than the responses reported earlier in turpentine inflammation (see eg. 4). In an attempt to increase the α_1-acid glycoprotein and sialyltransferase response to cytokine, the cytokine preparation from the adherent cell population was given to rats in three equal doses over the 36 hr period. Table 3 shows that this treatment increased the serum α_1-acid glycoprotein and sialyltransferase response although the response did not achieve that found in turpentine induced inflammation.

In a previous study (6) we identified Galβ1→4 GlcAcα2→6 sialyltransferase as a major acute phase reactant using lactose as substrate to produce isomers of sialyllactose. Fig. 1 shows that 6'(α2→6) and 3'(α2→3) sialyllactose were formed by liver, but the main or only product formed by serum was the 6'(α2→6) isomer with serum from the cytokine treated animals producing larger amounts of radioactive product. The identification of α2→6 sialyltransferase as the main acute phase reactant was confirmed using antiserum against rat hepatic Galβ1→4 GlcNAcα2→6 sialyltransferase. Table 4 shows that serum sialyltransferase activity following cytokine was largely eliminated by preincubation with 0.5 - 1.0 µl antibody prior to sialyltransferase assay; liver sialyltransferase activity was also reduced, but residual activity due to the presence of the other isoenzymes of sialyltransferase remained.

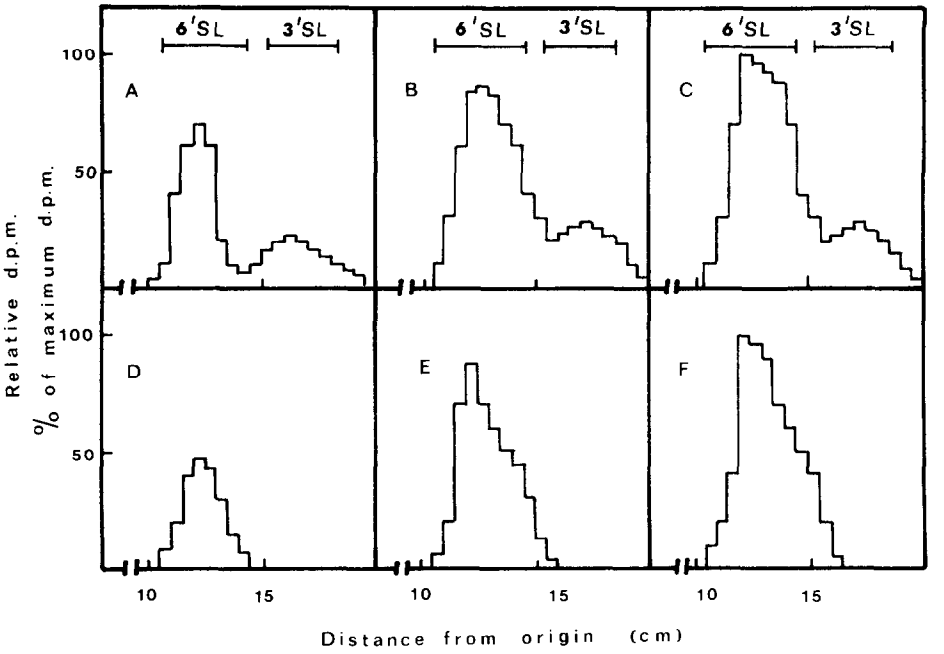

Fig. 1. Chromatograms showing sialyllactose isomers produced by serum and liver from control rats and those given cytokine from PEC or monokine from adherent cell populations. 6'SL shows the position of the 6'(NeuAcα2→6 Galβ1→4Glc) isomer and 3'SL shows the position of the 3'(NeuAcα2→3 Galβ1→4Glc) isomer. Chromatography was for 5 days (6) with 1 mg liver protein or 50 µl serum. Panels A, B and C; liver samples from control, PEC and adherent cell monokine, respectively; panels D, E and F, serum samples from control, PEC and adherent cell monokine, respectively. Results are presented as histograms as before (6) taking the maximum dpm from adherent cell monokine samples as 100%. The 100% value for liver was 22,000 d.p.m. and for serum 16,000 d.p.m.

DISCUSSION

The clearest evidence that monokines regulate the acute phase reactants are the increases that occur when hepatocytes are exposed to 25 - 30 kdalton

Table 4. Effect of Preincubation with Antiserum against Hepatic Galβ1→4 GlcNAcα2→6 Sialyltransferase on Serum and Liver Sialyltransferase from Rats Given Cytokine from PEC and Monokine from Adherent Cell Populations

vol antiserum µl	Sialyltransferase Activities+ d.p.m.			
	PEC		monokine	
	liver	serum	liver	serum
0	8650	3400	9541	3700
0.2	6450	1350	7500	1795
0.5	2500	320	3200	405
1.0	981	50	1051	75

+Results are expressed as d.p.m. [^{14}C] transferred from CMP-[^{14}C] N-acetylneuraminic acid in the standard assay with 250 µg asialo-α_1-acid glycoprotein as acceptor (see Ref. 6). Data given represent means from experiments with three rats. Reproducibility was within ±10%. Rats received single injections of 180 MCE PEC cytokine or 120 MCE adherent cell monokine.

monocyte-derived factors (14-27); hepatocyte-stimulating factor (HSF) is such a factor which has been partially characterized. Interleukin-I may also affect hepatocyte synthesis of acute phase proteins like amyloid A (25), but interleukin-1 does not regulate hepatocyte production of fibrinogen or albumin (22,23).

The studies reported here together with those described above lead to the conclusion that monokines play a major role in regulating the hepatic response of acute phase serum proteins. However, this report also supports the involvement of monokines as mediators of the response of two enzymes involved in glycoprotein metabolism. These are serum Galβ1→4 GlcNAcα2→6 sialyltransferase - a liver derived acute phase reactant (6) which also responds to HSF in hepatocyte culture (Woloski et al. unpublished) and hepatic β-N-acetyl hexosaminidase - a lysosomal enzyme which declines during injury (4). Both enzymes responded to monokine, but the N-acetylhexosaminidase also responded to cytokine from non-adherent cells. This suggests that several factors may be involved in the depression of hepatic glycosidases not all of which are of monocyte origin.

About 50 MCE of monokine were needed to elicit a good in vivo response of α_1-acid glycoprotein, sialyltransferase and albumin. This is about 100 times the level used to stimulate responses in hepatocyte cultures (15). However, considering the differences in the amount of tissue between intact animal and hepatocytes, coupled with the possibility of monokine inactivation in vivo, a 100 fold difference in dose between the two systems is not unreasonable. The observation that multiple injections of monokines caused larger in vivo responses would be consistent with the idea that there are in vivo mechanisms to inactivate the monokine. Inactivation in vivo could also explain the lower responses observed with monokine compared to turpentine injury (Table 3) where there would be a constant infusion of monokines into the circulation from activated monocytes located at the granuloma site.

ACKNOWLEDGEMENTS

This work was supported by Grant MA7685 from the Medical Research Council of Canada and Grant A5394 from the Natural Sciences and Engineering Research

Council of Canada. B.M.R.N.J.W. was the recipient of a Predoctoral Fellowship from the Faculty of Graduate Studies, University of Manitoba.

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