Acta Paediatrica Hungarica 29 (1–2), pp. 33–35 (1988–89)

Regulation of complement gene expression of murine macrophages: effects of histamine

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The complement system of mice is composed of twenty glycoproteins with outstanding importance in the immunological recognition and effector mechanisms [5]. Three complement proteins, C2, fB and C4 are encoded by genes within the major histocompatibility complex (MHC) on the short arm of chromosome 17, in mouse [1].

The major site of the production of complement components is the liver, but the macrophages are also able to express these proteins. In this study we checked the effect of histamine and its specific antagonists and agonists on the gene expression of C2, fB and C3 by murine peritoneal macrophages.

Materials and Methods

Mice

Seven- to ten-weeks-old male mice of CBA strains were purchased from The Jackson Laboratory (Bar Harbor, ME).

Media

RPMI 1640 (with or without methionine), Hanks' balanced salt solution (HBSS) without Ca and Mg and fetal calf serum were obtained from Gibco Laboratories (Grand Island, NY).

Cell culture

Peritoneal cells were harvested from peritoneal washings by cold HBSS, without Ca and Mg. The collected cells were washed and purified by adherence. The cells were treated with histamine, H1 antagonist chlorpheniramine, H2 antagonist cimetidine, H1 agonist 2-pyridyl ethylamine 2HCl (PEA), H2 agonist impromidine for 24 hours. All reagents were gifts of Smith, Kline & French Research Ltd.

mRNA analysis

Isolation of total cellular RNA from tissues and peritoneal macrophages, the gel electrophoresis and Northern blotting were done as described earlier [2].

Probes

The cDNA clones for C2 [3], fB [6] and C3 (Kindly provided by dr. Takahashi, Japan) were used. The purified inserts were nick translated by P-32 labelled, dCTP (New England Nuclear, Boston, MA) and used for hybridization. The autoradiography films were evaluated by densitometry.

Results and Discussion

The amounts of specific mRNAs are shown on Figure 1. and the quantitative calculation based on densitometry is summarized on Table I. When compared to the 'baseline' control value (lane 1) histamine has a consi-

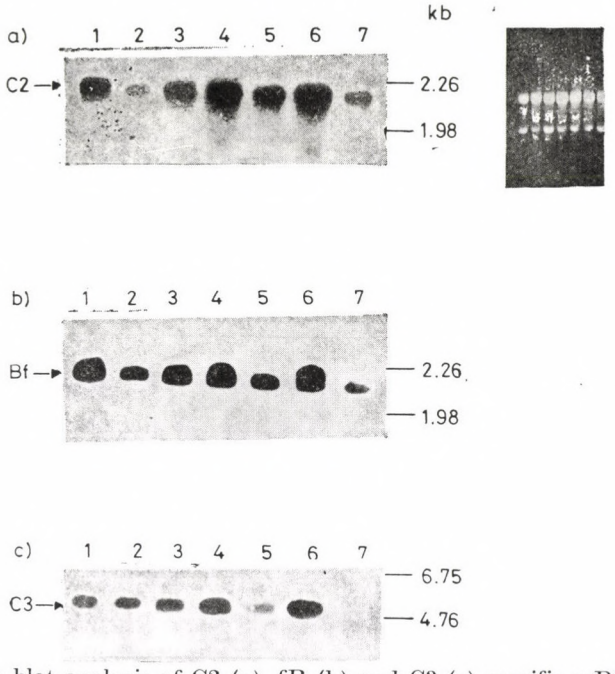

FIG. 1. Northern blot analysis of C2 (a), fB (b) and C3 (c) specific mRNA from mouse resident peritoneal macrophages incubated in the presence of medium (lane 1), 10^{-4} M (lane 2), 10^{-8} M (lane 3) histamine, 10^{-4} M histamine + 5×10^{-5} M Cimetidine (lane 4), 10^{-4} M histamine + 5×10^{-5} M chlorpheniramine (lane 5), 5×10^{-5} M PEA (lane 6) and 5×10^{-5} M impromidine (lane 7). Fifteen micrograms of total cellular RNA were loaded on the gels. UV light photograph of ethidium-bromide stained gel before transfer is displayed on the right

TABLE I

The amount of mRNAs evaluated by densitometry

	%		
	C2	fB	C3
Medium	100	100	100
Histamine 10 — 4 M	41	53	103
10 — 8 M	94	98	106
Histamine 10 — 4 M + cimetidine 5×10 — 5 M	109	97	167
Histamine 10 — 4 M + chloropheniramine 5×10 — 5 M	68	59	56
PEA 5×10 — 5 M	110	103	178
impromidine 5×10 — 5 M	47	44	29

derable down-regulation on the amount of C2 and fB mRNA (lanes 2 and 3). In both cases H2 antagonist (lane 4) but not the H1 antagonist (lane 5) can abolish the inhibition suggesting that histamine acted through H2 receptor. This conclusion is confirmed by the demonstration of the negative effect of H2 agonist (lane 7) while the H1 agonist (lane 6) revealed practically no effect.

Oppositely, histamine alone had virtually no effect on the quantity of C3 mRNA (lane 2, 3), but when it was added together with H1 antagonist, a down-regulation (lane 5) was detected. H2 agonist alone (lane 7) also inhibited the gene expression of C3. On the other hand, histamine + H2 antagonist (lane 4) or H1 agonist alone (lane 6) clearly enhanced the C3 gene expression.

The results show a down-regulation via H2 receptor of gene expression of all three complement component. Action of histamine on H1 receptor only affects that of C3 and possibly that counteraction is the reason why the histamine alone is virtually uneffective on C3 biosynthesis. When we checked the level of de novo synthetised C2, fB and C3 (data not shown) a similar tendency of effects were detected.

The stimulation of H2 receptor acts through the cyclic nucleotides and that of H1 receptor influences the Ca related intracellular mechanisms [4]. Our data demonstrate a definite difference between the effects on the gene expression of C3 initiated by this two intracellular signal transducing pathways.

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