Characterization and synthesis of a macrophage inhibitory peptide from the second constant domain of human immunoglobulin G

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We have shown that IgG hydrolysed by *Schistosoma mansoni* schistosomula inhibited various macrophage functions, especially phagocytosis and anti-schistosome cytotoxicity. Here we show that a tripeptide, Thr_{289}–Lys–Pro_{291}, of the second constant domain of human immunoglobulin G (peptide 286–292) reproduced the inhibitory effect of a total hydrolysate. Indeed the β-glucuronidase release from IgE–anti-IgE-stimulated rat and human macrophages decreased and its intracellular level did not rise after a prior incubation of the cells with Thr–Lys–Pro (500 nmol/ml). Moreover, the cell migration as well as the superoxide anion O_2^- generation were 50–80% reduced by the tripeptide. These results suggest that a single peptide set may be responsible for the decrease of the macrophage functions at the early stage of the parasite infection in the mammalian host. The pharmacologic properties of this tripeptide are under investigation.

Macrophage inhibitory peptide *Human IgG* *Second constant domain*

1. INTRODUCTION

IgG, bound onto the surface of *Schistosoma mansoni* schistosomula, was cleaved by proteases secreted by the larvae. This process of binding and cleavage of IgG molecules generates peptidic fragments, liberated in the larval environment, which interfere with various macrophage functions [1]. Indeed, it was initially shown that a total IgG hydrolysate inhibited the macrophage stimulation assessed by β-glucuronidase release and glucosamine incorporation, and reduced both phagocytosis of latex beads and IgE-mediated macrophage cytotoxicity against schistosomula [2]. The IgG hydrolysate apparently prevented the cell stimulation itself, without affecting its viability. IgE-activated macrophages being one of the main effector mechanisms involved in the anti-schistosome immunity, its inhibition by such peptides could, at least in part, facilitate host invasion by parasites. But more generally, the inhibiting effect on the phagocytic activity of the macrophage suggests the use of the active peptides as molecules with pharmacologic goals.

In the present study we have demonstrated that a tripeptide* from the second constant domain of human IgG reproduced the inhibiting effect of a total IgG hydrolysate on several macrophage functions.

2. MATERIALS AND METHODS

2.1. *Preparation of IgG hydrolysate*

Schistosomula of a Puerto-Rican strain of *Schistosoma mansoni* were obtained from cercariae by a mechanical procedure in vitro [3]. The larvae were incubated for 16 h at 37°C in MEM with 60 μg rat IgG/ml. The absence of bacterial contamination was controlled. After elimination

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of schistosomula by centrifugation, the hydrolysis was stopped by heating the supernatant 5 min at 100°C.

2.2. *Metabolic and functional parameters*

The extracellular release of the lysosomal β-glucuronidase, induced in IgE-stimulated cells by a further 30 min incubation with anti-IgE, was measured as in [4].

Superoxide anion O_2^- generation was quantified by the reduction of cytochrome *c* according to [5] as modified in [6] with IgE-stimulated cells triggered for 120 min by anti-IgE-opsonized zymosan in phenol-red free Hank's balanced salt solution (HW).

Specific IgE-dependent macrophage cytotoxicity against schistosomula was performed as in [7] by a 6 h-preincubation of the effector cells with *S. mansoni*-immune rat serum.

2.3. *Cell viability*

In all cases, >90% living cells were observed in the presence or absence of IgG peptides, estimated by the trypan blue exclusion test and the fluorescein diacetate method [8].

2.4. *Macrophage triggering by IgE*

For human alveolar macrophages, the free lung cells were recovered after informed consent from non-atopic patients, by bronchoalveolar lavage performed under fiberoptic bronchoscopy. The cell pellets of centrifuged lavage fluids, consisting of 93% alveolar macrophages and 7% lymphocytes, were resuspended in MEM with 10% foetal calf serum, and incubated for a 2 h-adherence phase. After removal of unadherent cells, the macrophages were 98% pure, and further incubated overnight in the same medium.

Rat peritoneal macrophages were purified to 95% by adherence, for 2 h, and incubated overnight like human alveolar macrophages.

Both cell populations were treated at 37°C with IgG peptides (500 nmol/ml) for 30 min, washed, and then exposed to IgE triggering [6,9]: a first 30 min-incubation at 37°C in 10 μg IgE/ml HW was followed, after washing, by a second 30 min-period in 10 μl anti-IgE antibody/ml HW. Supernatants were recovered, and cells lysed with Triton X-100 0.1% in Tris–$MgCl_2$ 0.01 M (pH 7.4) for extra- and intracellular lysosomal enzyme assay.

2.5. *Macrophage chemotaxis*

Rat peritoneal macrophages were purified to 95% by a 2 h-adherence phase, incubated for 30 min with IgG peptides, washed, and recovered by scrapping. The macrophage-enriched population was adjusted to 10^6 cells/ml MEM with 10% foetal calf serum, and used for chemotaxis. Macrophage migration was assessed at 37°C for 2 h in a 48-well microchemotaxis assembly (Neuroprobe Corporation, Bethesda MD), with a 5 μm porosity polycarbonate filter (Nuclepore Corporation, Pleasanton CA) [10] and 10% zymosan activated normal rat serum in MEM as chemoattractant. The filter was stained with Giemsa, and chemotaxis was quantified by counting and averaging the macrophages that migrated completely through the filter, and present on its lower surface.

2.6. *Peptides*

Peptide synthesis was performed by the solid phase method [11] in an automated Beckman synthesizer Model 990B as in [12]. *N*-Boc-amino acids were used and trifunctional amino acids were protected as *N*-Boc-Arg (Tos), *N*-Boc-Lys (Z) and *N*-Boc-Thr (Bal). Asn was incorporated with DCC/HOBT coupling as proposed in [13]. The protected peptidyl-resins were cleaved by a 1 h HF treatment in the presence of anisole. The crude peptides were first purified by gel filtration (G10, Pharmacia) followed by preparative reverse-phase chromatography (Lobar C_8, Merck). The purified peptides were checked by analytical HPLC, TLC in two different solvent systems and amino-acid analysis of their acid hydrolysates.

3. RESULTS

When the rat macrophages were incubated with various peptides of the second constant domain of human IgG before IgE triggering, a decrease of the selective lysosomal enzyme release (table 1) and of the chemotaxis (table 2) could be observed. Thr–Lys–Pro exhibited the maximum inhibitory effect. In contrast, the hexapeptide Asn–Ala–Lys–Thr–Lys–Pro was without effect on the macrophage activity, while the penta- and tetrapeptides showed intermediate activity. Furthermore, the tetrapeptide Tuftsin as well as its competitive inhibiting tripeptide Lys–Pro–Arg, had no effect on the trig-

Table 1

Release and intracellular level of β-glucuronidase from IgE–anti-IgE-stimulated rat peritoneal macrophages following incubation with IgG peptides of the second constant domain

First incubation	Second incubation	N[a]	Enzyme release[b]	% Inhibition	Intracellular[b] level
IgE	Anti-IgE	15	12.6 ± 0.9	0	241 ± 13
IgE + Asn–Ala–Lys–Thr–Lys–Pro[c]	Anti-IgE	2	15.6 ± 5.8	–	260 ± 32
IgE + Ala–Lys–Thr–Lys–Pro	Anti-IgE	4	11.1 ± 3.4	15	222 ± 33
IgE + Lys–Thr–Lys–Pro	Anti-IgE	10	10.6 ± 3.1	20	213 ± 27
IgE + Thr–Lys–Pro	Anti-IgE	13	5.4 ± 0.4	73	174 ± 16
IgE + Tuftsin: Thr–Lys–Pro–Arg	Anti-IgE	2	10.6 ± 0.2	20	244 ± 12
IgE + Lys–Pro–Arg	Anti-IgE	5	10.3 ± 0.8	23	247 ± 13
IgE + IgG hydrolysate	Anti-IgE	7	6.9 ± 0.7	58	185 ± 16
HW	HW	15	2.8 ± 0.4	100	204 ± 14

[a] Number of experiments in triplicate
[b] nmoles of hydrolysed substrate/10^6 cells (mean ± SEM)
[c] All peptides were used at 500 nmol/ml

Table 2

Chemotaxis of rat peritoneal macrophages after incubation with peptides of the second constant domain of IgG

Macrophages pre-incubated with	Number of cells[a]	% Inhibition
HW	44.1 ± 8.0	–
+Asn–Ala–Lys–Thr–Lys–Pro[b]	50.1 ± 8.9	–
+ Ala–Lys–Thr–Lys–Pro	27.8 ± 2.7	37
+ Lys–Thr–Lys–Pro	25.2 ± 4.0	43
+ Thr–Lys–Pro	6.6 ± 2.7	85

[a] Mean of 16 optic fields of experiments made in triplicate
[b] All peptides were used at the concentration of 500 nmol/ml

Table 3

Superoxide anion generation by IgE–anti-IgE-triggered rat peritoneal macrophages in the presence of IgG-peptides[a]

Preincubation with	Superoxide anion[b]	% Inhibition
Medium alone	71.7 ± 10.4	–
IgG hydrolysate	19.3 ± 9.1	73
Tripeptide (Thr–Lys–Pro)[c]	35.4 ± 1.06	51

[a] Triggering signal given by IgE (30 min) followed by anti-IgE opsonized zymosan
[b] nmoles of SOD-inhibitable reduced cytochrome $c/10^7$ cells
[c] Used at 500 nmol/ml

gering by IgE. Thr–Lys–Pro exhibited the same inhibitory effect on the superoxide anion O_2^- generation by rat macrophages, and reproduced the inhibition observed with an hydrolysate of IgG by schistosomulum-secreted proteases (table 3). This last observation may be related to the reduction of the IgE-dependent cytotoxicity expressed by rat macrophages against schistosome larvae after incubation with the tripeptide: in such conditions, macrophages exhibited a 38% decrease in the in vitro killing of schistosomula.

Besides its activity on rat macrophages, the same tripeptide proved to be inhibitory also for human alveolar macrophages during the interaction with IgE, either as myeloma protein or as allergen-specific antibody (table 4). For both rat and human macrophages, in addition to the inhibitory effects on enzyme exocytosis, the intracellular β-glucuronidase, in the presence of the tripeptide Thr–Lys–Pro, remained at the level of unstimulated cells when compared to optimally triggered phagocytes.

Table 4

Release of β-glucuronidase from IgE/anti-IgE and allergen-stimulated human alveolar macrophages after incubation with an IgG tripeptide of the second constant domain

First incubation	Second incubation	Enzyme release[a]	% Inhibition	Intracellular level
IgE	Anti-IgE	87.5 ± 8.0	0	1757.5 ± 6.8
+ Thr–Lys–Pro[b]	Anti-IgE	37.5 ± 3.0	71	1692.5 ± 37.0
HW	HW	17.5 ± 3.0	–	1668.2 ± 16.0
Serum from asthmatic patient	Allergen[c]	35.0 ± 0.5	0	875.0 ± 5.0
+ Thr–Lys–Pro	Allergen	22.5 ± 3.5	63	831.5 ± 7.5
HW	HW	15.0 ± 0.5	–	781.5 ± 40.5

[a] Results expressed as nmol substrate/10^6 cells
[b] Peptide used at 500 nmol/ml
[c] Allergen (*Dermatophagoides pteronyssinus*) used at 100 ng HW/ml

4. DISCUSSION

Here we show that peptides of the second constant domain of human IgG molecules strongly inhibited various macrophage activities during IgE–anti-IgE stimulation and, more particularly, the release of lysosomal enzymes such as β-glucuronidase, and the superoxide anion O_2^- generation. The cell migration was also significantly reduced. The inhibitory effect of the tripeptide Thr–Lys–Pro decreased upon the addition of amino acids to the NH2 position (Asn_{286}, Ala_{287} and Lys_{288}), or to the COOH position (Arg_{292}) of the IgG sequence. The peptide apparently interfered with the stimulation process itself, without affecting the basal cell activity. Indeed this effect could not be related to any direct toxic action on the cells, since appropriate controls clearly showed that macrophage viability was not altered. The tetrapeptide Tuftsin (Thr–Lys–Pro–Arg), known to increase the macrophage functions, had neither activating nor inhibiting effect on the rat macrophages when added during the IgE–anti-IgE triggering. This could be related to the high efficiency of the IgE–anti-IgE reaction to stimulate the macrophage [14]. In addition, Lys–Pro–Arg, which is a competitive inhibitor of Tuftsin [15], was without effect in these triggering and inhibitory processes. Therefore, to interfere with the macrophage activity, Thr–Lys–Pro probably does not exert its action through the Tuftsin receptors of the cells, and may use cellular targets distinct from these reported in the Tuftsin system. Moreover, Thr–Lys–Pro was never described as an inhibitor of Tuftsin.

Of particular interest was the interaction of this peptide with the triggering of human alveolar macrophages by the serum of asthmatic patients which was shown to induce a rapid and strong IgE-dependent stimulation of the cell metabolism and secretory functions upon the addition of specific allergens [6,16].

Though the effect of this tripeptide on the macrophage activity resembles that described for the whole hydrolysate of IgG by parasitic proteases, it is not excluded that other peptides from the IgG molecule could play such an inhibitory role, and their identification is presently under investigation. Nevertheless, the COOH-terminal proline of the tripeptide suggests the need of prolidase activity among the proteases secreted by the young larval stage of schistosome. Indeed, some of them exhibit prolidase and collagenase specificities [1].

An immunopharmacologic study of this tripeptide and of its analogues is underway.

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