Regulation of gelatinase B (MMP-9) in leukocytes by plant lectins

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Abstract The stimulatory or inhibitory effects of plant lectins on the production of gelatinase A (MMP-2) and gelatinase B (MMP-9) by mononuclear white blood cells was investigated by substrate zymography. Leukocyte cultures from 24-h old buffy coats were spontaneously activated and produced high levels of gelatinase B. Using such cultures the suppressing activity of the *Datura stramonium*, *Viscum album*, *Bauhinia purpurea*, *Triticum aestivum* and *Maackia amurensis* lectins on gelatinase B induction were demonstrated. When fresh leukocyte preparations from single blood donors were used, low levels of gelatinase B were produced. The induction of gelatinase B was confirmed for concanavalin A and phytohaemagglutinin (PHA-L4). In addition, the *Urtica dioica*, *Calystegia sepium*, *Convolvulus arvensis* and *Colchicum autumnale* lectins were documented as novel and potent inducers of gelatinase B. Since high circulating gelatinase B levels are associated with specific pathologies, including shock syndromes, the acute toxicity of many lectins might be partially mediated or influenced by gelatinase induction.

Key words: Gelatinase; Lectin; Lymphocyte; Monocyte; Endotoxin; Matrix metalloproteinase

1. Introduction

Many plant lectins become increasingly well understood in terms of molecular structure, physiological role and biological effects on mammalian cells. Since various types of plant lectins are potent toxins, data on lethality in mammalian hosts and some immunological effects have been documented [1–3]. Perhaps the best studied immunological aspect is the cell agglutinating property of many plant lectins. Agglutination of white blood cells and haemagglutinating activity have often been used as a read-out system during lectin purification procedures. Intrinsically connected with leukocyte agglutination is the mitogenic activity of particular plant lectins [4–7]. Due to the multivalent carbohydrate recognition, various lectins can cross-link cells and cell receptors and abolish mitotic quiescence which eventually results in the transformation of, for instance, lymphocytes into lymphoblasts [5]. The exact mechanism of this mitogenic signal is not known, but there exist a number of similarities with superantigen-mediated triggering of lymphocytes [4,5]. In brief, several lectins induce polyclonal activation of lymphocytes which results in proliferation and differentiation into cytokine-producing cells. For example, it has been documented that concanavalin A (Con A) and phytohaemagglutinin induce the cytokines IL-1, IL-6 and the chemotactic factor IL-8 and various other chemokines [8–10]. These two lectins thus possess various immunomodulating effects.

Leukocytes use various biochemical mechanisms to achieve their chemotactic movement towards the inflammatory focus. One important mechanism is the production of extracellular matrix metalloproteinases such as gelatinase A and gelatinase B [11]. These enzymes enable the producer cells to degrade the subendothelial and subepithelial basal membranes as well as the denatured collagens in connective tissues (after cleavage by collagenases). Gelatinase A (MMP-2) and gelatinase B (MMP-9) are constitutive and inducible enzymes, respectively, in inflammatory processes [12]. In humans, the former has a molecular mass of 65–75 kDa and is omnipresent in body fluids and secreted by most cell types. Regulation of gelatinase A production has been documented in specialized tissues such as the testis and the kidney [13,14]. The inducible gelatinase B has, depending on the species, on the producer cell type and the posttranslational processing, a molecular mass of 85–110 kDa. This enzyme activity increases in various autoimmune diseases such as rheumatoid arthritis and multiple sclerosis and has recently attracted a lot of attention as a possible target for the treatment of these diseases [15,16]. For gelatinase A, there is evidence that lectins can induce the activation of the zymogen form into the active enzyme form [13]. For gelatinase B there is limited information about induction of de novo synthesis or activation by lectins. As a first step to investigate the possible role of lectins as environmental factors in pathology, we have studied the regulation of gelatinases in human leukocytes. For this purpose a unique and broad panel of well characterized and novel plant lectins were compared for enzyme regulation.

2. Materials and methods

2.1. Cell purification and induction

Fresh or overnight stored heparinized buffy coats were obtained from the Red Cross Transfusion Centre of Antwerp, Belgium. Leukocytes were fractionated by gradient centrifugation ($400\times g$, 30 min) on Lymphoprep (Nycomed, Life Technologies) and the mononuclear cell fraction (90% purity) was seeded at 5×10^6 cells/ml. In a further step monocytes were isolated either by adherence (1 h, at 37°C and followed by two washes with serum-free medium) or by positive selection with anti-CD14. The latter purification procedure was by incubation (30 min at 4°C) with paramagnetic microbeads conjugated with a monoclonal antibody against CD14 and cell isolation using the VarioMACS system (Miltenyl Biotec, Bergisch, Germany). Cell purity was analysed by FACS and reached at least 80% for the anti-CD14 sorted cells. Inductions were done in 24-well plates using serum-free cell culture medium (1 ml/well) to which the lectins were added in concentrations ranging from 10 ng/ml to 100 μg/ml. After incubation at 37°C for various time intervals, the cell culture supernatants were collected and usually 10–20-μl samples were sufficient for zymography or cytokine analysis.

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2.2. Lectins

The following lectins [2] were used in this study (the international nomenclature for abbreviations is used throughout): *Bauhinia purpurea* lectin (BPA), soybean (*Glycine max*) agglutinin (SBA), *Wisteria floribunda* agglutinin (WFA), *Dolichos biflorus* agglutinin (DBA), *Datura stramonium* lectin (DSA), *Urtica dioica* agglutinin (UDA), *Amaranthus caudatus* lectin (ACA), wheat germ (*Triticum aestivum*) agglutinin (WGA), *Maackia amurensis* agglutinin (MAA), *Viscum album* chitin-binding agglutinin (VisAlbCBA), pea (*Pisum sativum*) lectin (PSA), *Vicia faba* lectin (VFL), *Rhizoctonia solani* agglutinin (RSA), *Colchicum autumnale* agglutinin (CAA), *Tulipa* lectin (TxLC-I), *Phaseolus vulgaris* agglutinin E (PHA-E4), *Phaseolus vulgaris* agglutinin L (PHA-L4), *Allium sativum* agglutinin (ASA), *Allium ursinum* agglutinin (AUA), *Galanthus nivalis* agglutinin (GNA), *Narcissus pseudonarcissus* agglutinin (NPA), *Hippeastrum* hybrid agglutinin (HHA), *Xanthosoma sagittifolium* agglutinin (XSA), *Listera ovata* agglutinin (LOA), *Calystegia sepium* agglutinin (Calsepa), *Convolvulus arvensis* agglutinin (Conarva), *Helianthus tuberosus* agglutinin (Heltuba). All lectins were electrophoretically pure [2,3,17–19] and prepared as a stock solution of 1 mg/ml. Ten-fold dilution series were made in 0.9% pyrogen-free NaCl solution. Con A was purchased from Calbiochem.

2.3. Zymography

Gelatin zymography was done as previously detailed [20]. Briefly, for molecular weight standardization commercially available protein standard mixtures were included (Bio-Rad, SDS-PAGE standard) and for the identification of gelatinases A and B a laboratory control of recombinant mouse gelatinase B, expressed in *Pichia pastoris* was used [21]. After development of the enzymatic activity, scanning densitometry was used to quantify the gelatinolytic activity. Significant induction or inhibition of gelatinase B was evidenced in at least two independent induction experiments.

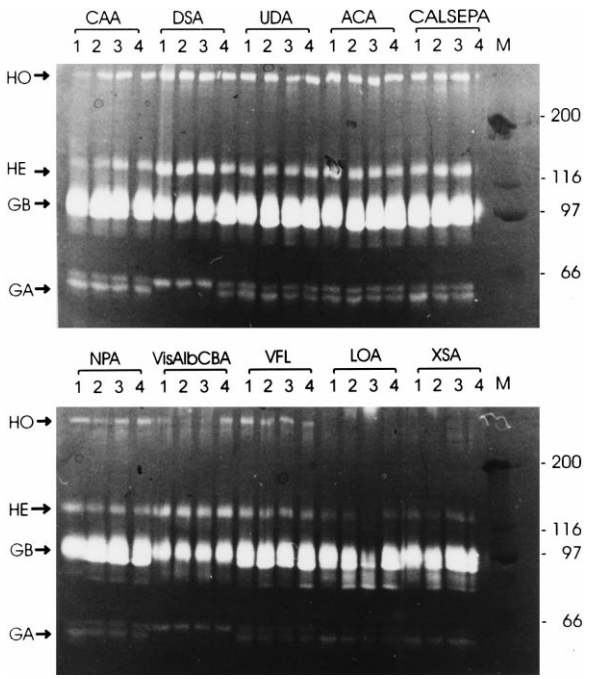

Fig. 1. Spontaneous gelatinase production in aged buffy coats. Mononuclear cells, prepared from 24 h old blood collections were seeded at 3×10^6 cells in 4 ml culture medium with 2% fetal bovine serum and stimulated for 24 h with various lectins. The lanes contain 20-μl samples stimulated with CAA, DSA, UDA, ACA, Calsepa, NPA, VisAlCBA, VFL, LOA and XSA, respectively. The samples in lanes 1, 2, 3 and 4 were induced with the individual lectins at 100, 10, 1 and 0.1 μg/ml, respectively. The lanes M contain molecular weight markers which are indicated in kDa. At the left side gelatinase A (GA), gelatinase B (GB), the heterodimer with neutrophil gelatinase B-associated lipocalin (HE) and the gelatinase B homodimer (HO) are indicated by small arrows. When the cells are cultured under serum-free conditions, gelatinase A is not detectable. Control cell cultures treated with medium only yielded similar zymography pictures.

3. Results

3.1. Gelatinase B inhibition by lectins in mononuclear cells

In a number of experiments leukocytes were prepared after overnight storage either from pooled or individual human buffy coats. It was a constant finding, as measured in more than 50 different cell isolations, that these pooled or aged culture fluids contained gelatinase B. When such leukocyte cultures were used, induction with lectins did not enhance the production of gelatinase B activity (Fig. 1 and data not shown). However, such cell cultures with high background levels were useful to show on zymography analysis the dominant presence of gelatinase B over gelatinase A and its heterogeneity. In some instances the zymography showed the 92.2-kDa monomer, the 110-kDa heterodimer of gelatinase

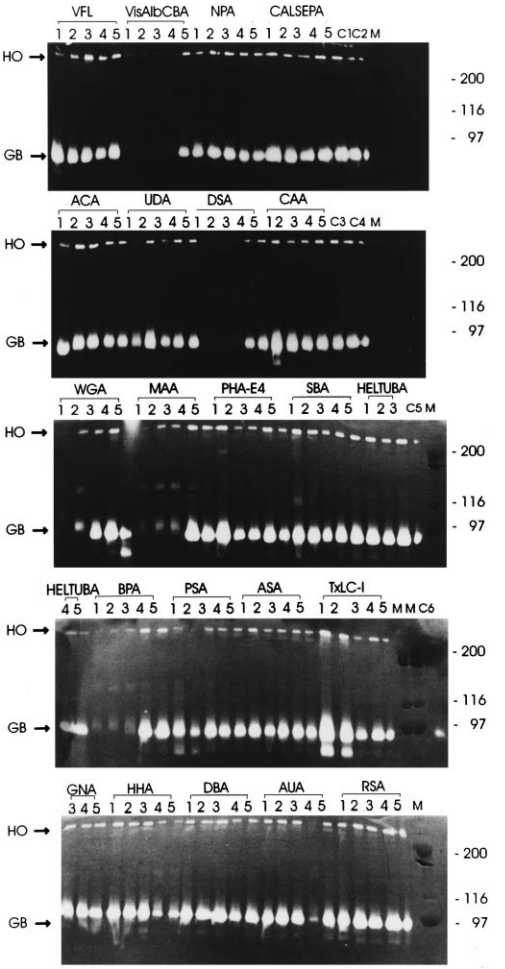

Fig. 2. Inhibition of gelatinase B production in fresh leukocytes. Mononuclear cells were induced under serum-free conditions. All the lanes contain 20-μl samples induced with, respectively, VFL, VisAlbCBA, NPA, Calsepa, ACA, UDA, DSA, CAA, WGA, MAA, PHA-E4, SBA, Heltuba, BPA, PSA, ASA, TxLC-I, GNA, HHA, DBA, AUA and RSA. The cultures in lanes 1, 2, 3, 4 and 5 were treated with 100 (for HHA: 81.6 μg/ml), 10, 1, 0.1 and 0.01 μg/ml, respectively. Lanes C1 to C6 contain control samples from unstimulated cells, whereas the lanes M show the molecular weight markers in kDa. At the left side, gelatinase B (GB) and its homodimer (HO) are indicated.

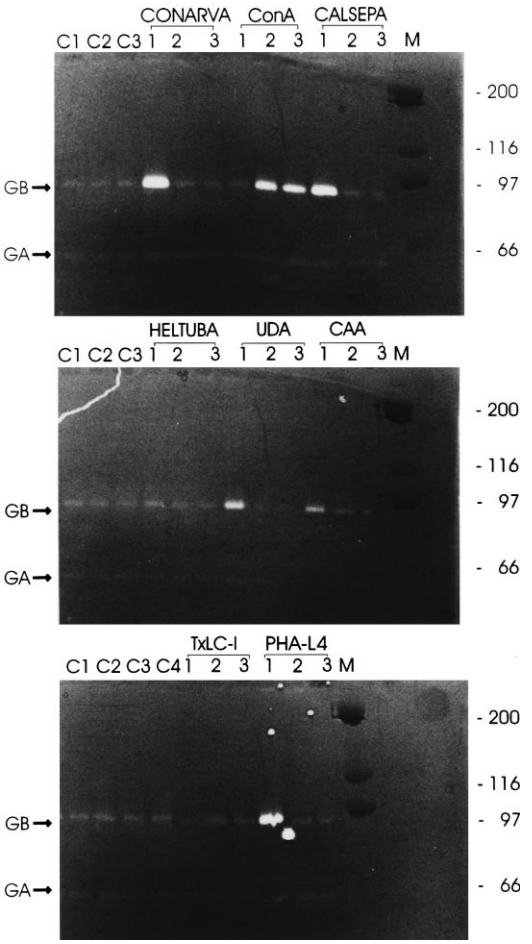

Fig. 3. Induction of gelatinase B production in fresh buffy coats. Mononuclear cells from a fresh buffy coat were isolated and seeded at 5×10^4 cells per ml. After 44 h supernatants were collected and 20-μl samples analysed by zymography. Lanes C1–3 contain samples from control cells. The samples in the other lanes were induced with, respectively, Conarva, Con A, Calsepa, Heltuba, UDA, CAA, TxLC-I and PHA-L4. Lanes 1, 2 and 3 show inductions with the lectins at 100 (for L4: 50 μg/ml), 1 and 0.01 μg/ml, respectively. In lanes M the molecular weight markers are indicated in kDa. The arrows GA and GB indicate the migration of gelatinases A and B, respectively.

B with neutrophil gelatinase B-associated lipocalin and a 200-kDa homodimer [22]. The inhibition of gelatinase B production by lectins can be shown by these preparations although the inhibitory effect is more pronounced when fresh leukocyte preparations were used. For instance, in Fig. 1 *Viscum album agglutinin* (VisAlbCBA) reduced the levels of gelatinase B monomer and dimer, whereas *Datura stramonium lectin* (DSA) inhibited the levels of the gelatinase B monomers. The latter two lectins also inhibited gelatinase B release from fresh mononuclear cells (Fig. 2). In addition, Fig. 2 shows that *Maackia amurensis* (MAA), wheat germ agglutinin (WGA) and *Bauhinia purpurea* (BPA) lectins inhibited significantly gelatinase B induction. In some instances, induction or activation to lower molecular weight forms was observed. For instance, tulip lectin (TxLC-I) dose-dependently induced synthesis or activation of gelatinase B (Fig. 2).

3.2. Gelatinase B induction by lectins in mononuclear cells

To visualize gelatinase B induction it was essential to reduce background levels of the enzyme in the unstimulated cultures. This was achieved by using lower cell numbers from fresh cell cultures under serum-free conditions. Serum, however, contains the constitutive enzyme gelatinase A and might be a helpful marker to standardize inducibility of gelatinase B. Under serum-free cell culture conditions, the constitutive production of the gelatinase A marker enzyme depended on the cell type used. Fibroblasts, monocytes and granulocytes produce low, very low and no gelatinase A, respectively (data not shown and [12]). Furthermore, it was essential that the human leukocytes were freshly isolated, immediately purified and induced. Fig. 3 illustrates the inducibility of gelatinase B by various lectins on mononuclear leukocytes. The lectins from *Convolvulus arvensis* (Conarva), *Calystegia sepium* (Calsepa), *Urtica dioica* (UDA), *Colchicum autumnale* (CAA), Con A and phytohaemagglutinin from *Phaseolus vulgaris* (PHA-L4) induced gelatinase B in mononuclear leukocytes. However, under these particular experimental conditions tulip lectin (TxLC-I) failed to induce gelatinase B.

4. Discussion

The aim of the present study was to document whether and which lectins alter the expression of gelatinases in human leukocytes. Classical activation mechanisms for cytokine and enzyme production by lymphocytes and monocytes include cytokines and direct cell–cell contacts [9,11,20,23,24]. Lectins have also been described as potent immunomodulators by inducing lymphocyte activation and cytokine production [4,5]. The secretion of metalloproteinases by lymphocytes and monocytes has been documented for a restricted number of lectins [12,23,25]. Gelatinase B has recently gained attention as a possible target in autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis [15]. Many autoimmune disorders are classified as multifactorial diseases since no single etiological agent has yet been detected and because both genetic (e.g. human leukocyte antigens, HLA) and environmental factors might contribute to these disease states. So far, mainly microbial agents have been studied as environmental factors, though dietary factors may be relevant too. It is possible that vitamins [26,27] or for instance lectins, which are often stable and protease-resistant dietary proteins, might contribute to disease promotion by regulation of the protease load [15]. The presence of lectin-specific antibodies in serum illustrates that lectins and/or lectin peptides are absorbed by the host. Whether such intestinal absorption can lead to pharmacological levels, as in the present study, remains, however, to be shown.

Two observations are here documented. A number of lectins induce gelatinase B, whereas others seem to decrease the induced production of gelatinase. The reasons for high gelatinase B activity in cell preparations of pooled buffy coats and 24-h aged cultures from single donors are trivial. It is known, indeed, that direct cell–cell contacts, such as those occurring in mixed leukocyte reactions, and small amounts of contaminating endotoxin, present in many serum preparations, induce the production of gelatinase B [20,23,25]. Therefore, lower cell numbers and serum-free conditions were used. Serum, however, contains the constitutive enzyme gelatinase A which is a helpful marker to standardize inducibility of gelatinase B. Under serum-free cell culture conditions, the constitutive production of the gelatinase A marker enzyme depended on the

cell type used. Fibroblasts, monocytes and granulocytes produce low, very low and no gelatinase A, respectively (data not shown and [12]). Furthermore, it was essential that the human leukocytes were freshly isolated, immediately purified and induced. Since on zymography analysis the 'spontaneously' induced gelatinase B in some experiments is clearly of neutrophilic origin ([22] and Fig. 1), we postulate that the used gelatinase B inhibitory lectins may primarily act on the secretion instead of on the de novo synthesis by neutrophilic granulocytes. Granulocytes contain considerable amounts of gelatinase B, prepacked in specific granules and ready for immediate secretion. The lectins which inhibited neutrophil gelatinase B secretion also inhibited gelatinase B production in mononuclear cell preparations. According to the results shown in Fig. 2 the inhibition of gelatinase B activity is not exclusively determined by the nominal carbohydrate-binding specificity of the lectins. Most but not all chitin-binding lectins are inhibitory (VisAlbCBA, DSA and WGA vs. UDA). Similarly, of all GalNAc-specific lectins tested only BPA exhibited an inhibitory activity. Finally, the Neu5Aα(2,3)GalNAc-specific MAA also clearly inhibited the gelatinase B activity.

Urtica dioica and *Colchicum autumnale* lectins, as well as *Calystegia sepium* and *Convolvulus arvensis* lectins are documented as novel potent gelatinase B-inducing lectins. With the latter lectins, comparable or even higher induction levels were observed than those obtained with Con A and phytohaemagglutinin. The demonstration of the gelatinase B-inducing activity of UDA, CAA, Calsepa and Conarva clearly illustrates that lectins with a different carbohydrate-binding specificity are capable of enhancing signal transduction pathways leading to the same enzyme activity in human lymphocytes. In addition, the fact that not all lectins with the same nominal specificity induce gelatinase B (e.g. UDA vs. VisAlbCBA, DSA and WGA) suggests that the receptor glycans have a highly specific and complex structure.

Recently, we illustrated the induction of gelatinase B in septicaemia and speculated that endotoxin-induced release of gelatinase B in the systemic circulation might constitute a mechanism contributing to the development of shock syndromes [28]. Since some lectins induce similar levels of gelatinase B release from blood cells as those obtained by LPS, the acute toxicity of these lectins might be partially caused by such a mechanism.

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