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Inflammatory and anti-inflammatory effects of soybean agglutinin

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Abstract

Soybean agglutinin (SBA) lectin, a protein present in raw soybean meals, can bind to and be extensively endocytosed by intestinal epithelial cells, being nutritionally toxic for most animals. In the present study we show that SBA (5-200 µg/cavity) injected into different cavities of rats induced a typical inflammatory response characterized by dose-dependent exudation and neutrophil migration 4 h after injection. This effect was blocked by pretreatment with glucocorticoid (0.5 mg/kg) or by co-injection of N-acetyl-galactosamine (100 x [M] lectin), but not of other sugars (100 x [M] lectin), suggesting an inflammatory response related to the lectin activity. Neutrophil accumulation was not dependent on a direct effect of SBA on the macrophage population since the effect was not altered when the number of peritoneal cells was increased or decreased *in vivo*. On the other hand, SBA showed chemotactic activity for human neutrophils *in vitro*. A slight increase in mononuclear cells was observed 48 h after *ip* injection of SBA. Phenotypic analysis of these cells showed an increase in the $CD4^+/CD8^-$ lymphocyte population that returned to control levels after 15 days, suggesting the development of an immune response. SBA-stimulated macrophages presented an increase in the expression of CD11/CD18 surface molecules and showed some characteristics of activated cells. After intravenous administration, SBA increased the number of circulating neutrophils and inhibited in a dose-dependent manner the neutrophil migration induced by *ip* injection of carrageenan into peritoneal cavities. The co-injection of N-acetyl-galactosamine or mannose, but not glucose or fucose, inhibited these effects. The data indicate that soybean lectin is able to induce a local inflammatory reaction but has an anti-inflammatory effect when present in circulating blood.

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- Soybean agglutinin (SBA)
- Neutrophil migration
- Inflammation

Introduction

By virtue of their specific carbohydrate-binding properties, lectins have been widely used as laboratory tools in biology. Galactosyl-binding lectins are of particular interest. These lectins have been identified in many animal cells as membrane components of macrophages, lymphocytes and hepatocytes (1-3), and also as soluble proteins in various organs of many species (4). The functional roles of these proteins have been investigated, and many of them seem to participate in important immune and physiopathologi-

cal events (4-6). Cell-specific carbohydrate recognition has been shown to mediate, at least in part, the adhesion to vascular endothelial cells and the migration of leukocytes to inflamed tissues (6). Recently, a galactose-binding lectin released by activated macrophages was identified as a specific neutrophil attractant *in vivo* and *in vitro* (7). Thus, to study the protein-carbohydrate interaction involved in leukocyte migration during the inflammatory response it would be useful to identify exogenous lectins that present characteristics similar to and mimic the effects of endogenous lectins.

Soybean (*Glycine max*) agglutinin (SBA), a specific N-acetyl-galactosamine/galactose-binding lectin, is considered to be one of the major factors responsible for the low nutritional value and deleterious effect of raw soybean meal. SBA and other lectins can bind to and be extensively endocytosed by epithelial cells of the small intestine, being toxic for most animals (8). It has been suggested that the binding of these toxic lectins to receptors on the intestinal brush borders helps to stabilize these proteins against proteolytic breakdown, thus facilitating their endocytosis. As a consequence, damage in the microvilli of the epithelial cells may occur, leading to antinutritional and toxic effects (8). On the other hand, several studies have demonstrated interesting immunebiochemical applications of SBA *in vitro* such as the separation of B and T lymphocytes (9), increased bone marrow cell proliferation, the stimulation of monocyte/macrophage colony growth (10), and marking the late stages of macrophage differentiation (11). However, apart from investigations devoted to nutritional aspects, few studies have been carried out on the *in vivo* effects of SBA. In this report, by focusing on the phenomenon of leukocyte migration, we demonstrate and characterize the inflammatory reaction locally induced by SBA, as well as its anti-inflammatory effect after systemic administration.

Material and Methods

Animals

Male Wistar rats weighing 150-180 g and 6-8-week old inbred C57-black/6 mice were housed in temperature-controlled rooms and received water and food *ad libitum* until use. In all experiments the animals were treated in accordance with published regulations for animal experiments.

Lectin

Soybean agglutinin was purchased from Sigma Chemical Co. (St. Louis, MO) and dissolved in sterile phosphate buffered saline (PBS) or saline, pH 6.8, just before use. After dilution, SBA presented an agglutinating activity of 16 µg/ml for fresh human blood group A erythrocytes and 62.5 µg/ml for fresh rat blood erythrocytes after 1-h incubation at 25°C with a 2% cell suspension.

Drugs and chemicals

All drugs and reagents, unless otherwise stated, were obtained from Sigma. BN52021, the PAF antagonist, was kindly provided by Dr. P. Braquet, Institute Beaufour (France).

In vivo migration of neutrophils and mononuclear cells

Peritoneal cavities. SBA (50-200 µg) diluted in 3 ml sterile PBS was injected intraperitoneally (*ip*) into rats. At different times, the cells were harvested by lavage of the cavity with 10 ml PBS supplemented with 5 IU/ml heparin and 0.1% (w/v) BSA. Usually 70-80% of the lavage fluid was recovered. Animals injected with PBS alone were used as controls. Total and differential cell counts were performed as described elsewhere (12). The results are reported as number of cells per ml of collected fluid.

Pleural cavities. SBA (5-50 µg) diluted in 100 µl sterile saline was injected intrathoracically (*it*) into rats. After 4 h, leukocytes were harvested from the pleural cavities by lavage with 3 ml PBS, as described elsewhere (13). The volume of fluid recovered from the lavage was measured in order to evaluate the increase in vascular permeability induced by lectin injection. The exudate volume (µl) is defined as the difference between the volume harvested from SBA-treated and control (saline-injected) animals. Leukocyte migration is reported as the number of cells per cavity.

Air pouches. Rat skin air pouches were produced as previously described (13). SBA (100 µg) diluted in 1 ml sterile PBS was injected into the 6-day old pouches. After 4 h, cells were harvested by lavage of the cavity with 10 ml PBS, and neutrophil migration was evaluated. Results are reported as number of cells per cavity.

In order to check a possible contamination of SBA with bacterial lipopolysaccharide (LPS), in some experiments the lectin was pre-incubated for 1 h at 37°C with polymyxin (50 µg/ml) just before injection and tested for the ability to induce neutrophil migration.

In some experiments the animals were pretreated subcutaneously with the following drugs 1 h before SBA administration: dexamethasone (0.5 mg/kg), BN52021 (20 mg/kg), nordihydroguaiaretic acid (NDGA, 100 mg/kg), and indomethacin (5 mg/kg). For other experiments SBA was dissolved in a solution of N-acetyl-ß-D-galactosamine (NAG) or mannose (Man) before being injected into the cavities. Carbohydrates were used at concentrations 100 times greater on a molar basis than that of the lectin. Animals treated with the carbohydrates alone were used as controls.

Changes in peritoneal cell population

Increase in the number of peritoneal macrophages. A group of rats was injected with 10 ml thioglycollate (Tg) broth (3% w/v). After three days, these animals (Tg group) were injected *ip* with 100 µg SBA. Neutrophil migration was evaluated 4 h later and compared with that obtained with non-pretreated animals (N group).

Depletion of the peritoneal cell population. Rat peritoneal cavities were washed with sterile saline as described elsewhere (13), causing a significant depletion (90%) of the peritoneal cell population. Control (sham) rats were manipulated in the same way but no fluid was injected or withdrawn. Peritoneally washed rats (W group) or sham-operated rats (S group) were injected (*ip*) with 100 µg SBA and neutrophil migration was estimated 4 h later.

In vitro neutrophil chemotaxis migration assay

Assays of *in vitro* neutrophil migration were performed as described elsewere (7) in a 48-well chemotactic microchamber (Neuroprobe, Cabin John, MD). Human neutrophils were isolated from heparinized peripheral blood of healthy human volunteers using mono-poly resolving medium (Flow Lab., Rockville, MD). The cells were then washed and suspended in RPMI 1640 medium containing 0.1% (w/v) BSA (RPMI-BSA) to provide 10^6 neutrophils/ml. Purified neutrophils were placed in the upper chamber and the test samples dissolved in RPMI-BSA or medium alone were placed in the lower chamber. The peptide FMLP (0.1 µM) was used as the reference chemoattractant. The number of cells that migrate through the entire thickness of the 5-µm polycarbonate filter (Millipore Corp., Bedford, MA) during 1-h incubation at 37°C in a 5% CO_2 atmosphere was counted. At least five fields were counted for each assay and each sample was assayed in triplicate. The results are reported as mean ± SEM number of neutrophils per field.

Immunocytochemical identification of mononuclear cells

Mononuclear cells harvested from mouse peritoneal cavities injected with 100 µg SBA 24 h, 48 h, 72 h, 96 h, 15 days and 30 days before were assayed for their properties in expressing Thy^+, $CD4^+$, CD8 and CD11/CD18 surface antigens. Antigen expression was determined by an indirect binding assay using the primary antibodies (ATCC/USA; diluted 1:2), G-7 (anti-Thy), GK-1.5 (anti-CD4), H-35 (anti-CD8) and 5C6 (anti-CD11b/CD18, Mac1). A peroxidase-conjugated anti-rat IgG diluted 1:100 was used as secondary antibody. Cells were then stained with hematoxylin and peroxidase-positive cells were counted (total 200 cells) under a light microscope. Results are reported as percentage of Thy^+, $CD4^+$, $CD8^+$ and $CD11/CD18^+$ ($Mac1^+$).

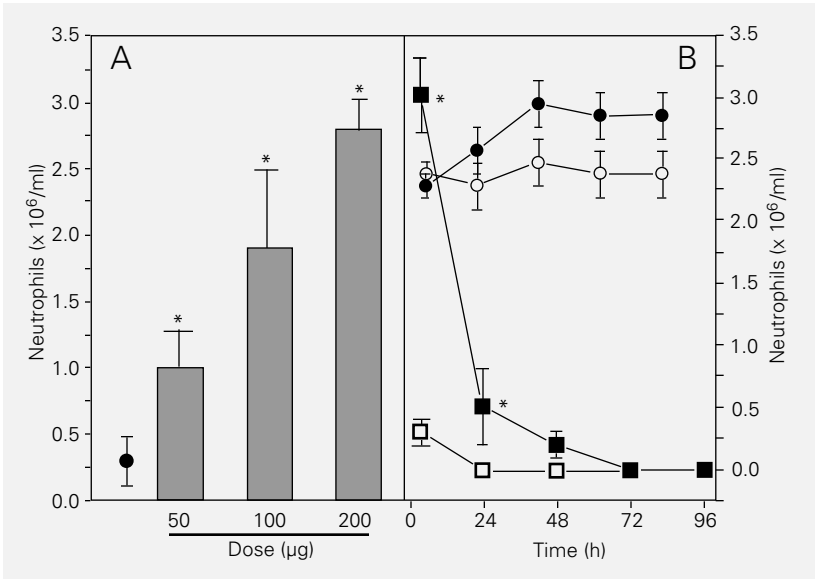

Figure 1 - Neutrophil migration induced by SBA injected into rat peritoneal cavities. *Panel A*, Different doses of SBA were injected *ip* into rats and the neutrophil migration (gray bars) was evaluated 4 h later. Animals injected with sterile PBS (closed circle) were used as controls. *Panel B*, Time-course of neutrophil (squares) and mononuclear cell (circles) accumulation into rat peritoneal cavities induced by SBA (200 µg; closed symbols). Animals injected *ip* with sterile PBS (open symbols) were used as controls. Results are reported as the mean ± SD of at least 6 animals. *P<0.05 compared to the control group (ANOVA followed by the Bonferroni *t*-test).

Effect of intravenous injection of SBA on carrageenan-induced neutrophil migration

Male rats were injected (*iv*) through the penile vein with a sterile SBA solution (50-200 µg in saline), 200 ng LPS (from *E. coli*, 026:B6) or 200 µl sterile saline alone. In some experiments carried out to check a possible contamination of SBA with LPS, the drugs and vehicle were pre-incubated for 1 h at 37°C with polymyxin (50 µg/ml) before injection. In other experiments SBA was dissolved as described earlier in a solution of NAG, Man or glucose (Glu) before *iv* administration. Thirty minutes after this treatment, the animals received an intraperitoneal injection of carrageenan (Cg, 500 µg/3 ml). Neutrophil migration into peritoneal cavities was evaluated 4 h later, as described above.

Statistical analysis

Statistical significance was assessed by ANOVA followed by the Bonferroni *t*-test and $P<0.05$ was considered to be statistically significant.

Results

SBA-induced neutrophil migration into different cavities

Figure 1 shows that 4 h after *ip* injection SBA induced significant neutrophil accumulation into the peritoneal cavities of rats. This effect was dose-dependent (Figure 1A) and the number of neutrophils returned to control levels after 48 h (Figure 1B). In contrast, at this time only a slight increase of mononuclear cells occurred which was maintained up to 96 h (Figure 1B, filled circles).

An intense neutrophil accumulation occurred in rat pleural cavities after *it* administration of SBA (Figure 2A). A dose-dependent increase in the pleural exudate volume was observed in parallel (Figure 2B). The

chemotactic effect for neutrophils was also evident when the lectin (100 µg) was injected into the dorsal air pouches of rats. The number of neutrophils in this cavity rose from 1.8 ± 0.2 (controls) to 12.1 ± 1.5 x 10^6 cells/ml (SBA) 4 h after lectin injection. SBA-induced neutrophil migration into the peritoneal (Figure 3A) and pleural cavities (Figure 3B), as well as into the subcutaneous air pouches of rats (data not shown) was significantly inhibited by pretreatment of the animals with dexamethasone. Pretreatment of the animals with a PAF antagonist (BN52021) or with the cyclo-/lipoxygenase (dual) inhibitor, NDGA, partially inhibited the neutrophil migration induced by *ip* injection of 200 µg SBA (SBA = 2.6 ± 0.6; BN52021 = 1.51 ± 0.2; NDGA = 1.17 ± 0.18 x 10^6 neutrophils/ml). In contrast, the specific cyclooxygenase inhibitor, indomethacin, did not modify the effect of the lectin on the accumulation of neutrophils in the rat peritoneal cavity (indomethacin = 2.5 ± 0.7 x 10^6 neutrophils/ml). Furthermore, NAG but not Man blocked the neutrophil accumulation in all cavities studied, when simultaneously injected with SBA (Figure 3). The injection of carbohydrate alone gave values similar to those obtained for controls (PBS). Pre-incubation of SBA with polymyxin at doses that efficiently reduced (75%) LPS-induced neutrophil migration (300 ng, *ip*) did not reduce the chemotactic effect of the lectin (data not shown).

Influence of peritoneal cell population on SBA-induced neutrophil migration

As described earlier, when rats were pretreated with Tg a significant increase in the number of peritoneal macrophages occurred (from 1.8 ± 0.1 to 4.0 ± 0.8 x 10^6 cells/ml). On the other hand, a marked reduction in resident peritoneal cell number (>95%) was observed in animals subjected to peritoneal lavage. SBA was injected into the peritoneal cavities of saline-washed (W) or Tg-pre-

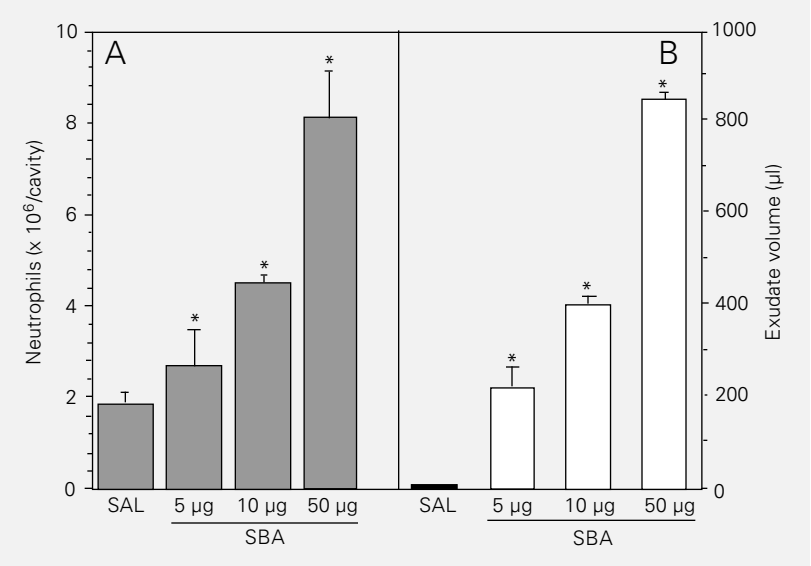

Figure 2 - Effect of injection of SBA into pleural cavities of rats. *Panel A*, Neutrophil migration was evaluated 4 h after *it* injection of SBA or sterile saline (SAL). *Panel B,* Exudate volume elicited by SBA 4 h after *it* injection. Animals injected with sterile saline, used as control, did not present an exudate in their pleural cavities. Results are reported as the mean ± SD of at least 6 animals. *P<0.05 compared to saline-injected animals (ANOVA followed by the Bonferroni *t*-test).

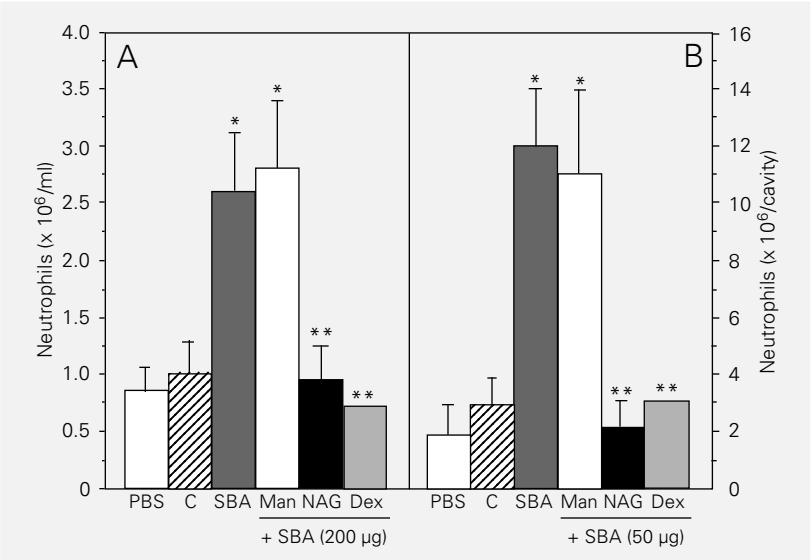

Figure 3 - Inhibitory effect of dexamethasone and carbohydrates on SBA-induced neutrophil migration in rats. *Panel A*, Neutrophil migration into peritoneal cavities. *Panel B*, Neutrophil migration into pleural cavities. Animals were pretreated with dexamethasone (Dex, 0.5 mg/kg; *sc*) 1 h before SBA injection (*ip*: 200 µg/cavity; *it*: 50 µg/cavity). In other experiments SBA was simultaneously injected with N-acetyl-galactosamine (NAG) or mannose (Man). Control animals were injected with PBS or with carbohydrates (C) alone. Neutrophil migration was evaluated after 4 h. Results are reported as the mean ± SD of at least 6 animals. *P<0.05 compared to PBS-injected animals (ANOVA followed by the Bonferroni *t*-test). **P<0.05 compared to animals injected with SBA alone.

treated animals and the neutrophil migration was evaluated 4 h later. The neutrophil chemotactic effect of SBA was not affected by peritoneal lavage (W group: $3.6 \pm 0.4 \times 10^6$ cells/ml) or by an increase in macrophage population (Tg group: $2.4 \pm 0.2 \times 10^6$ cells/ml) when compared to control animals (S group: 3.2 ± 0.5 and N group: $2.5 \pm 0.2 \times 10^6$ cells/ml).

In vitro neutrophil migration induced by SBA

SBA, at concentrations ranging from 0.5 to 5 µM, showed an *in vitro* chemotactic activity for human neutrophils similar to that observed for FMLP (10 nM) used as a reference chemoattractant. The results of a typical experiment were as follows: medium (RPMI-BSA) = 19.7 ± 3.7 neutrophils/field; FMLP = 65 ± 18 neutrophils/field, and SBA: 0.17 µM = 25 ± 1; 0.5 µM = 65 ± 9.3; 1.67 µM = 49 ± 10 and 5 µM = 39 ± 15 neutrophils/field.

Immunocytochemistry of mononuclear cells from SBA-stimulated cavities

Forty-eight h after SBA injection (100 µg) into the peritoneal cavities of rats, a slight increase in the number of mononuclear cells occurred. A similar effect was also observed in mice, which were used throughout the experiments to investigate the phenotype of the mononuclear cells. Table 1 shows that 48 h after SBA injection the Thy^+ lymphocyte population increased from 12% (controls) to 35%. This pattern was maintained until the 15th day, decreasing to control levels after 30 days. Most of this lymphocyte population was $CD4^+/CD8^-$. Furthermore, 48 h to 96 h after injection of the lectin there was a significant increase in the expression of the surface adhesion molecule integrin $CD11b/CD18^+$ ($Mac1^+$) of the macrophage population when compared to cells harvested from animals treated with saline.

Table 1 - Immunophenotypes of SBA-stimulated mononuclear cells from mouse peritoneal cavities.

Mononuclear cells were harvested from SBA (100 µg)-stimulated (S) or saline-injected (C) mouse peritoneal cavities after different times. Antigen expression was determined by immunocytochemical methods. The results are reported as percentage of the total number of mononuclear cells. Data represent the mean of 5-6 animals. The standard deviation for any group was about ±3%. *P<0.05 compared to the respective control group (C) (ANOVA followed by the Bonferroni *t*-test).

Group	24 h		48 h		72 h		96 h		15 days		30 days	
	C	S	C	S	C	S	C	S	C	S	C	S
Thy^+	12	3*	13	35*	12.6	38*	13	31*	12	35*	11.6	2*
$CD4^+$	-	-	-	31*	1.2	35*	-	38*	-	35*	-	-
$CD8^+$	-	-	-	-	-	-	-	-	-	-	-	-
$Mac1^+$	8	13	8	31*	10	42*	9	40*	9	12	9	9

Effect of intravenous injection of SBA on neutrophil migration

Figure 4A shows the dose-dependent inhibitory effect of SBA (50-200 µg, *iv*) on neutrophil migration induced by an intraperitoneal injection of carrageenan (Cg) in rats. The intravenous administration of SBA (200 µg) 30 min before the inflammatory stimulus inhibited by about 75% the migration of neutrophils into rat peritoneal cavities that were challenged with Cg. This effect was similar to that obtained with LPS (200 ng, *iv*) used as positive control in a similar protocol. The analysis of blood cell alterations showed that 4 h after *iv* injection of SBA the number of circulating neutrophils was highly increased when compared to animals injected with PBS (*iv*): PBS = 7.4 ± 3.5 and SBA = $24.5 \pm 3.4 \times 10^6$ cells/ml. When the lectin was simultaneously injected with the carbohydrates NAG or Man, the inhibitory effect of SBA on neutrophil migration was reversed (Figure 4B). The inhibitory effect was not modified by the co-administration of SBA with glucose (Figure 4B) or fucose (data not shown). Incubation with polymyxin B did not modify to a large extent the ability of SBA to decrease neutrophil migration

(from 75% to 68%). However, pre-incubation of LPS with polymyxin before *iv* administration significantly decreased from 80% to 37% the potent inhibitory effect of endotoxin on neutrophil migration induced by Cg.

Discussion

In the present report we showed that soybean lectin, one of the major factors responsible for the toxicity of raw soybean meals, induced an acute inflammatory reaction when injected into different rat cavities. This reaction was characterized by intense neutrophil migration and fluid extravasation and was inhibited by pretreatment with glucocorticoids and, to a lesser extent, by a PAF antagonist or by lipoxygenase inhibitors. This effect was completely blocked when the lectin was simultaneously injected with NAG, the specific binding sugar for SBA. Mannose, a nonspecific binding carbohydrate, did not alter this effect. These data strongly suggest that neutrophil accumulation induced by SBA is dependent on its specific carbohydrate-binding properties, that probably activate leukotrienes and PAF biosynthesis *in vivo*, which may account for its migratory effect. Interactions between leukocytes and endothelial cells by homologous carbohydrate recognition domains have been shown to mediate the adhesion and probably the migration of leukocytes from blood to tissues during inflammation (14). The selectins are a family of adhesion proteins expressed on leukocytes and endothelium which are responsible for the rolling of neutrophils along the surface of the venular endothelium (6). These proteins are natural ligands for carbohydrate moieties of glycoproteins to which they bind via their lectin domain (6,15). A macrophage-derived neutrophil chemotactic factor (MNCF) (16), a new cytokine with lectin-like properties (7), has been identified and characterized. MNCF released by macrophages stimulated with different stimuli (LPS, cytokines and some plant proteins) (13,16,17) induced neutrophil migration *in vivo* in animals pretreated with glucocorticoid (16,17). The chemotactic effect of MNCF is inhibited by D-galactose (7). Our data do not indicate whether SBA acts through the release of MNCF. First, in contrast to MNCF, the effect of SBA is inhibited by dexamethasone. Second, in contrast to LPS and other stimuli (13,16,17), the neutrophil migration induced by SBA was not modified by increasing or decreasing the number of macrophages in peritoneal cavities. *In vitro* experiments showed that SBA was not able to induce the release of MNCF from rat macrophage monolayers (data not shown). Moreover, the migratory effect of SBA on neutrophils was not attributable to LPS contamination since polymyxin B did not change the SBA response. On the other hand, this lectin was able to induce mast cell

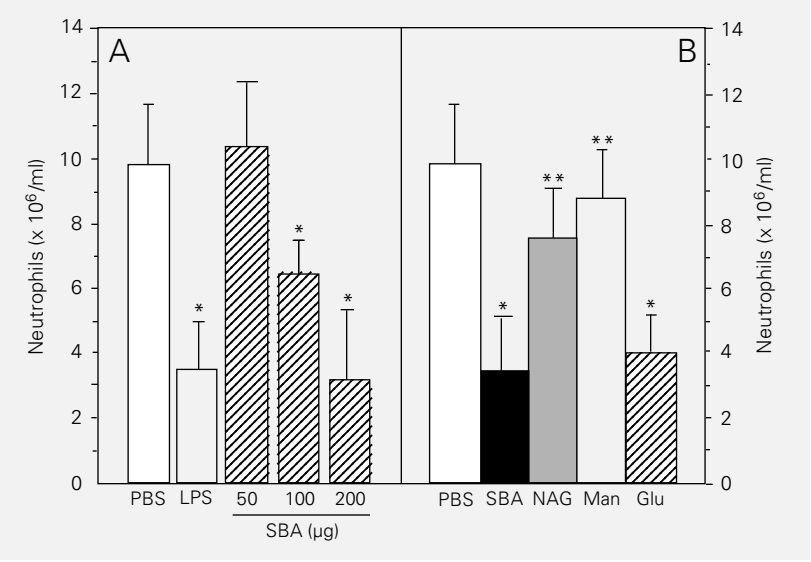

Figure 4 - Effect of intravenous injection of SBA on neutrophil migration induced by carrageenan injected into rat peritoneal cavities. *Panel A*, Carrageenan (Cg, 750 µg/cavity) was administered *ip* to rats 30 min after intravenous injection of SBA (50-200 µg), LPS (200 ng) or PBS (200 µl). *Panel B*, SBA (200 µg) was simultaneously injected with N-acetylgalactosamine (NAG), mannose (Man) or glucose (Glu) by the intravenous route, 30 min before *ip* injection of Cg. Neutrophil migration was evaluated after 4 h. Carbohydrates diluted in PBS were injected *iv* and did not interfere with the effect of Cg. Results are reported as the mean ± SD of at least 6 animals. *P<0.05 compared to animals injected *iv* with PBS (ANOVA followed by the Bonferroni *t*-test). **P<0.05 compared to animals injected *iv* with SBA alone.

degranulation *in vitro* (data not shown). Thus, mast cell activation is a possible pathway that may also contribute to the SBA-induced inflammatory response. However, since the inhibition of PAF and leukotriene pathways did not cause a complete blockade of the neutrophil accumulation induced by the lectin, other mechanisms such as the induction of cytokine production or even a direct chemotactic effect of SBA may account for neutrophil migration. In fact, SBA tested at concentrations from 0.5 to 5.0 µM showed an *in vitro* chemotactic activity for human neutrophils that was comparable to that observed for 0.1 µM FMLP (data not shown). After *ip* administration of SBA to mice, only a discrete increase (20%) in the number of mononuclear cells occurred. However, a critical change in the phenotype of this population was observed. In contrast to the controls, SBA-treated animals presented a long-lasting increase in the $Thy^+/CD4^+/CD8^-$ lymphocyte population. It has been reported that SBA does not have a significant mitogenic activity *in vitro* (18). However, our data suggest that, in the course of the SBA-induced inflammatory response, this lectin may trigger the development of an immune response, probably inducing lymphocyte migration or proliferation *in viv*o and altering the balance between lymphocyte and macrophage populations in the cavity. Moreover, macrophages from SBA-stimulated cavities presented an increased expression of CD11b/CD18 surface integrin, a characteristic that indicates macrophage activation. Actually, these cells showed other signs of activation such as an increase in the spreading index on a glass surface and in the enzymatic intracellular pool (N-acetyl galactosidase), and an increased capacity to produce nitric oxide in the presence of LPS, when compared to resident macrophages (Benjamin CF and Barja-Fidalgo C, unpublished results).

One of the most interesting nutritional aspects of SBA is its interaction with intestinal epithelial cells leading to extensive endocytosis and to systemic absorption of the lectin by the small intestine (8). Therefore, we investigated the possible interference of SBA in the systemic circulation with the acute inflammatory response induced by carrageenan. Intravenous injection of SBA caused a dose-dependent inhibition of neutrophil migration induced by the irritant stimulus. This effect was blocked when the lectin was simultaneously injected with NAG or mannose, but not by other sugars. Since this lectin is a glycoprotein containing mannose in its structure (19), it is possible that besides the binding to galactosyl residues, SBA may also interact with endogenous lectins present in various tissues (2) through its own carbohydrate moiety. Probably, both types of interactions are important for the anti-inflammatory effect of SBA. It is important to note that any untoward effect on blood cells, such as *in vivo* hemagglutination and neutrophil aggregation or sequestration, did not contribute to the anti-inflammatory effect of SBA. After *iv* injection of the lectin, the erythrocyte count did not change and significant neutrophilia was observed (data not shown). In addition, the ability of some plant lectins to induce *in vitro* and *in vivo* the release/production of cytokines has been described (20). Several cytokines, in addition to their pro-inflammatory effects, also exhibit anti-inflammatory activities (21-23). Thus, the mechanisms underlying the inhibitory effect of SBA on neutrophil migration may be modulated *in vivo* by cytokines produced by lectin-stimulated cells. On the other hand, we cannot rule out a possible direct effect of SBA mediated by a lectin-carbohydrate binding, which could interfere with leukocyte rolling or adhesion to the endothelium. The precise mechanism by which SBA inhibited neutrophil migration to the inflammatory site is currently under investigation.

In summary, our data indicate that soybean lectin is able to induce an inflammatory

reaction when injected locally, which may contribute to its harmful effect on the small intestine. However, when SBA is present in the blood circulation, an inhibitory effect on neutrophil migration was observed, suggesting an anti-inflammatory effect.

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