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ARTICLE

# Effect of Intraperitoneally Administered Plant Lectins on Leukocyte Diapedesis and Visceral Organ Weight in Rats and Mice

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**The effects of intraperitoneally administered plant lectins were examined in rats and mice. Intraperitoneally injected ConA transiently decreased the leukocyte count in the peritoneal cavity, due to the agglutination and attachment of cells to the peritoneal lining. Subsequently the total cell count was increased for hours, exceeding initial values. Peritoneal fluid aspartate transaminase (AST) concentration showed little change during the accumulation of ascitic fluid. The most marked histological alterations were found when wheat germ lectin was injected ip. (WGA, 10 mg/kg, 6 h). Neutrophil granulocytes migrated across the wall of both arterioles and venules, but the response was highly variable among adjacent vessels. The wall of the arterioles may have impeded the migration of neutrophil granulocytes, resulting in their accumulation in the muscular layer. Granulocyte accumulation was also observed in patches under the mesothelium and in other sites of the interstitium. Marked dilatation and thrombosis of a few venules were also observed. Kidney bean lectin (PHA) induced similar but less pronounced changes. The neutrophil diapedesis suggests the release of mediator(s) from mesothelial cells and/or peritoneal white cells. The cytokine-induced neutrophil chemoattractant CINC-1, injected as control, resulted in the diapedesis of predominantly mononuclear cells in the omentum within 40 minutes. In rats ip. injected ConA increased the wet weight of spleen and liver within 6 and 10 h, respectively, but kidney weight did not change. Intravascular clumping of red blood cells, thrombosis and organ weight changes also suggest the absorption of ConA into the circulation. The experiments show that plant lectins, used as models of bacterial lectins, can reproduce some aspects of peritonitis.** (Pathology Oncology Research Vol 13, No 2, 139–143)

*Key words:* lectin, ConA, WGA, PHA, neutrophil, diapedesis, mesothelial, CINC

## *Introduction*

Toll-like receptors (TLR) of the innate immune system bind pathogen-associated molecular patterns (PAMPs), induce inflammatory responses and assist in the recognition of non-self molecules for adaptive immunity.[1] Bacterial carbohydrates are bound by lectins, mannose-binding protein (MBP) being the best known. Conversely, lectins on the tips of bacterial fimbria or pili adhere to cell surface glycoproteins. In a series of experiments we tried to separate the specific effects of lectins from those of the bacterial cell as a whole.

The small amount of fimbrial lectins thwarted their isolation; therefore, we used plant lectin models for the study of their biological effects. In earlier studies we demonstrated that some of the orally administered plant lectins stimulate intestinal secretion of water and electrolytes.[2,3] The same lectins when injected intraperitoneally bind to mesothelial and immune cells[4,5] and induce copious secretion of ascitic fluid.[6-9] In the present work, we have examined the effects of ip. injected lectins on tissues beyond the peritoneal lining.

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## *Materials and Methods*

### *Reagents*

WGA,[10] SBA[11] and PHA[12] were purified by affinity chromatography. The tetrameric PHA contained both E and L polypeptide chains. The specificities of the lectins are shown in *Table 1*. ConA, CINC-1 and BSA were Sigma products.

***Table 1.*** **The plant lectins used in the experiments**

| *Abbreviation* | *Source* | *Latin name* | *Carbohydrate specificity* |
|---|---|---|---|
| ConA | jack bean | *Canavalia ensiformis* | glucose/mannose |
| WGA | wheat germ | *Triticum vulgare* | sialic acid-β(1,6)Gal-GlcNAc |
| SBA | soybean | *Glycine max* | N-acetyl-galactosamine |
| PHA | kidney bean | *Phaseolus vulgaris* | complex specificity* |

*Not inhibited by simple sugars
Gal = galactose; GlcNAc = N-acetyl-glucosamine

### *Animals and protocol*

Female NMRI mice (24-25 g) and female Wistar rats (120-125 g) were used. All animals were of SPF quality. The experiments were approved by the local ethical committee and the Veterinary Office of Somogy County. In the majority of experiments six animals were used per group and three mice for histology. Test substances were dissolved in physiological saline and injected ip. in a volume of 0.1 ml. BSA was used as control protein. At different times the ether-anesthetized animals were decapitated and bled. The abdominal cavity was opened and the ascitic fluid was collected by an automatic pipette. In another experiment, the visceral organs of ConA-treated rats were removed and weighed.

### *Cell counts*

Peritoneal white cells were counted in a Bürker chamber, and the cell count was related to the total volume of ascitic fluid recovered. Control samples were obtained by washing the peritoneal cavity with one ml of physiological saline. In parallel studies smears of peritoneal cells were stained with Giemsa dye and observed under light microscope.

### *Chemical measurements*

For the hemagglutination assay, blood taken from the tail of untreated mice into a heparinized tube was centrifuged, and the red blood cells separated. The cells were washed three times with physiological saline, pipetted into the wells of a hemagglutination plate in 1:25 dilution and used to demonstrate the presence of free lectin in the ascitic fluid. The activity of aspartate transaminase (AST) was measured from the supernatant of the centrifuged ascitic fluid with a Cobras Mira apparatus.

### *Histology*

Tissue samples were taken from the abdominal wall, the omentum and the processus xyphoideus, fixed in 8% formaldehyde, embedded in paraffin and stained with hematoxylin and eosin. Microphotos were taken with a Zeiss AxioCam MRc5 camera.

### *Statistics*

Significance of differences was calculated with ANOVA or t-test of the SPSS program.

## *Results and Discussion*

Both purified ConA and the one present in the ascitic fluid poorly agglutinated the murine red cells. In contrast, ip. injected PHA (25 mg/kg) could be detected in the ascitic fluid for up to 12 h by hemagglutination. Traces of PHA, absorbed from the gut into the circulation could be detected in the body fluids for several days by immune reaction, a more sensitive method than the agglutination assay (E. Lavelle, personal communication).

Ascites was induced in rats by ip. injection of PHA (20 mg/kg). Although the total AST activity of ascitic fluid markedly increased during the first 6 h (not shown), little variation of AST concentration was observed for up to 16 h (*Fig. 1*). These findings suggest that the appearance of AST in the peritoneal cavity was due to a secretion of protein-rich ascitic fluid, and not to tissue damage.

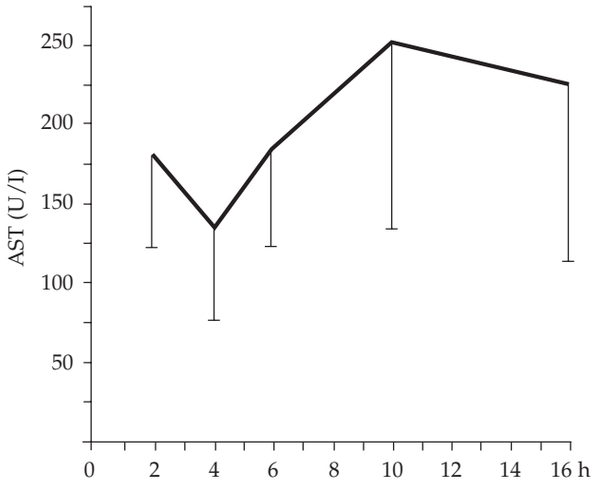


***Fig. 1.*** *Concentration of AST activity in the ascitic fluid of rats induced by ip. injection of 20 mg/kg PHA (N = 6). Significant difference ($p < 0.05$) was found between 4 h and 10 h values only.*

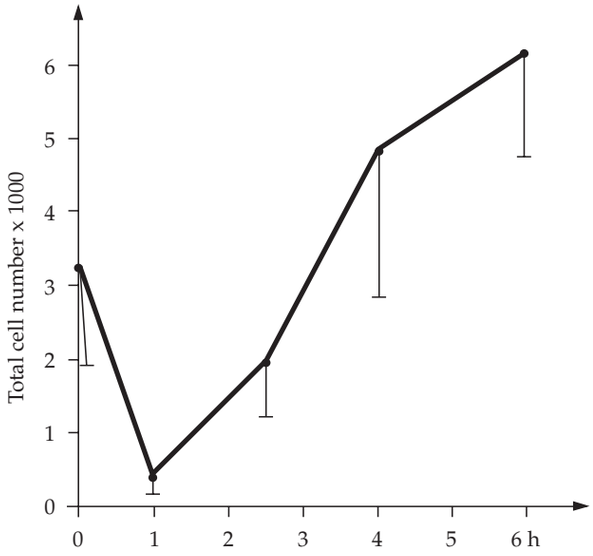


***Fig. 2.*** *Total number of white cells in the murine peritoneal fluid after inducing ascites by ip. injection of 25 mg/kg ConA (N = 6). The control value (0 h) was determined from 1 ml peritoneal lavage fluid. Differences are significant (p < 0.05), except those between 0 and 2.5 h.*

In experiments with mice ascites was induced by the ip. injection of ConA (25 mg/kg), and the peritoneal white cells were counted. During the progression of ascites, cell counts were expressed per volume of peritoneal fluid. Because of the small volume of peritoneal liquid in untreated mice, control values were obtained from 1 ml lavage fluid. The marked decline of cell counts 1 h after the injection (*Fig. 2*) was due to the agglutination of the cells and their attachment to the peritoneal lining, as observed microscopically and by histology (*Fig. 3c*). This made the 1 h counts unreliable. Thereafter, the free-floating cells reappeared. Although the concentration of the cells did not change significantly between 2 and 6 h, the total number of white cells steadily increased and exceeded the initial values (*Fig. 2*). The newcomer cells in the ascitic fluid were mostly neutrophil granulocytes. After 6 h the high viscosity of the ascitic fluid made accurate counting impossible.

The diapedesis of white cells from the circulation to the ascitic fluid was also examined using histological sections from lectin-treated mice. The process started with the attachment of the granulocytes to the vascular endothelium (*Fig 3e*). The most intense reaction was observed with WGA (10 mg/kg, 6 h) in samples taken from the ventral side of the processus xyphoideus. This site could not be reached by the injecting needle, excluding the possibility of an artefact. PHA (50 mg/kg) and especially ConA and SBA had less pronounced effects.

In WGA-treated mice the most striking observation was the mass of neutrophils in the swelled muscular layer of arterioles (*Fig. 3a*), which apparently formed a temporary barrier to cell migration, resulting in the accumulation of cells. The reaction of venules was less marked, due to their thinner wall. Collection of neutrophils could be observed in patches at a distance from the peritoneum or immediately under it (*Fig. 3b*), on their way into the ascitic fluid.

Affected and unaffected vessels were observed next to each other, which may be explained by local effects, e.g. the attachment of white cell aggregates to the peritoneum (*Fig. 3c*). Compact aggregates of red cells were observed in some of the vessels (*Fig. 3a*) and thrombosis was noted in a few dilated venules (*Fig. 3f*), possibly due to the direct effect of the absorbed lectin. This is in accordance with the known platelet-activating effect of lectins.[13]

Human mesothelial cells express glycosyl receptors for several lectins[5] and produce interleukin-8,[14] whose rodent homologue is the cytokine-induced neutrophil attractant (CINC). Several cytokines and inflammatory agents induce the production of cellular adhesion molecules (CAMs) on the surface of the vascular endothelium.[15] These molecules recognize, tether and ultimately bind circulating leukocytes, which open intercellular gaps for their migration into the surrounding tissues.[16] The different species of leukocytes use different, but overlapping molecular clusters for binding and diapedesis. Similar mechanisms may be operating in the metastasis of tumor cells.

In the present experiments we considered CINC as the primary candidate for the mediation of WGA-induced neutrophil attraction. When 5 μg CINC-1 was injected ip. into the mouse, for comparison with WGA, extravasation of predominantly mononuclear cells was observed (*Fig. 3d*). In contrast, masses of neutrophil granulocytes appeared after WGA treatment (*Fig. 3a*). However, the two experiments could not be performed under the same conditions; the rapid effect and degradation of CINC necessitated the termination of the experiment at 40 minutes, whereas the WGA challenge lasted for 6 h.

Lectin-binding was not sufficient for the massive neutrophil response; it was the specific effect of WGA, which recognizes a complex terminal moiety (including sialic acid) in glycoproteins. It appears to be a coincidence that both leukocyte and endothelial selectins (CAM molecules) are lectins that recognize sialic acid-containing molecules, but with a much narrower specificity than WGA does.[17]

***Table 2.*** **Effect of ip. ConA treatment (40 mg/kg) on rat organ weights**

| | *Liver* | *Spleen* | *Kidneys* |
|---|---|---|---|
| Control | 3.69±0.22 | 0.30±0.04 | 0.99±0.07 |
| ConA 6 h | 3.94±0.12 | 0.35±0.02* | 0.96±0.04 |
| ConA 10 h | 4.10±0.22* | 0.36±0.03* | 0.98±0.07 |

Wet weights are expressed in % of body weight. *significant difference (p < 0.05) from control

Although more work is required to clarify the mechanism of action of WGA, the present experiments show that WGA selectively attracts neutrophil granulocytes. This may provide a means for the study of neutrophil diapedesis without the interference of other species of leukocytes or the need for the experimental induction of tissue damage.

In ConA-injected rats the wet weight of spleen and liver increased within 6 or 10 h, respectively (*Table 2*), a numer-

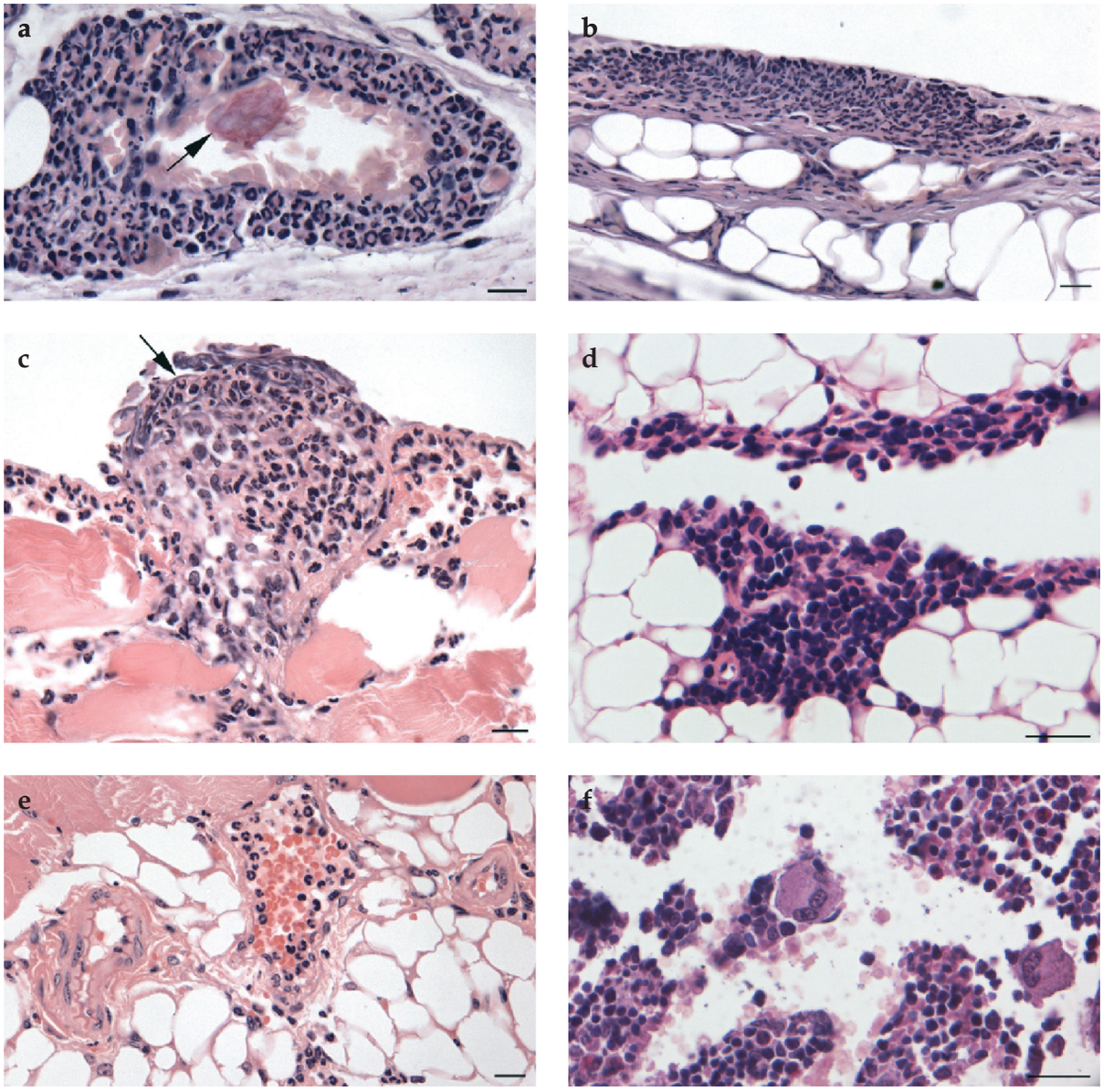


***Fig. 3***. *Histological alterations in mice injected ip. with lectins (HE). The bars correspond to 20 μm.* ***(a,b)*** *Ventral side of the processus xyphoideus of a mouse, 6 h after ip. injection of 10 mg/kg WGA.* ***(a)*** *An arteriole with a mass of neutrophil granulocytes in the muscular layer, while the interstitium is free of white cells. In the lumen of the vessel a red cell aggregate is seen (arrow), but most of the erythrocytes are either free-floating or attached to the intima.* ***(b)*** *A subperitoneal dense accumulation of leukocytes.* ***(c)*** *An aggregate of white cells attached to the peritoneal lining of the murine abdominal wall, 4 h after the ip. injection of 50 mg/kg PHA. The arrow indicates the layer of mesothelial cells under the attachment. The submesothelial area is densely infiltrated with leukocytes.* ***(d)*** *Omentum of a mouse 40 minutes after ip. treatment with cytokine-induced neutrophil chemoattractant (CINC-1), showing accumulation of mononuclear cells.* ***(e)*** *Neutrophil granulocytes attached to the intima of a venule, between two slightly affected arterioles in the murine abdominal wall, 4 h after ip. injection of 50 mg/kg PHA.* ***(f)*** *A thrombus containing widely different cell types, mostly mononuclear cells. Ventral side of the processus xyphoideus of a mouse, 6 h after ip. injection of WGA (10 mg/kg).*

ically small but statistically significant difference ($p < 0.05$). It is known that these organs remove serum protein precipitates and red cell aggregates from the circulation, and iv. administered ConA may induce hepatitis.[18] Lectins are not excreted by the kidneys and kidney weight did not change during the experiment.

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