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INFLAMMATORY CUTANEOUS REACTION INDUCED BY THE LECTIN OF *DIOCLEA GRANDIFLORA* (MART.)

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The lectin from Dioclea grandiflora *(Mart.) that selectively binds glucose and mannose, when subcutaneously injected in mouse induces an inflammatory cutaneous reaction whose histological analysis reveals an hemorrhagic ulceration with exudative reaction accompanied by an influx of polymorphonuclear leukocytes and giant cells. The presence of lymphocytes and plasma cells in the lesion was insignificant. In order to characterize the* in vivo *action of inflammatory factors generated by this lesion, distinct lines of mice were used: high and low antibody responder mice; the genetically selected mice to the acute phase of inflammatory reaction; lines of mice deficient in C5, a protein of the complement system. It is shown that the lectin of* D. grandiflora *acts as an inflammatory agent probably promoting exocytosis and release of mediators.*

Key words: lectin – *Dioclea grandiflora* lectin – inflammatory reaction – cutaneous lesion

Cells are coated with carbohydrate and related molecules that can be specifically attached to lectins. This specific association results in a variety of roles for this particular class of molecules that are increasingly used in biological research (Lis & Sharon, 1985; Etzler, 1986). The ability of concanavalin A (ConA), a lectin from Jack beans, to induce histamine release from basophils and mast cells (Siraganian & Siraganian, 1974; Ennis et al., 1981) and its capacity to promote platelet activation with secretion (Santoro, 1983) are well known properties. Although these meditors play a role in the inflammatory reaction, only recently lectins have been reported to act as inflammatory agents. This is the case with the inflammatory reaction induced by ConA in the skin of rabbits with a biphasic influx of neutrophils (Colditz & Cibulsky, 1987) and also with the apparent increase in tuberculin potency by a factor of a thousand on its inflammatory effect (Marchal et al., 1986). Still related to inflammation, lectins have been used as probes to differentiate diagnosis of tumor from inflammation (Kojima & Jay, 1987) or to detect microheterogeneity of alpha-1-antichymotrypsin in sera from patients with various inflammatory syndromes (Hachulla et al., 1988; Breborowicz & Mackiewicz, 1988; Pos et al., 1990).

In the present study we examined the evolution of a cutaneous inflammatory reaction induced by the lectin of *D. grandiflora* seeds that selectively binds glucose and mannose. In order to characterize the *in vivo* action of inflammatory factors generated by this lectin as well as the participation of the Complement system, distinct lines of mice were used: High (H_{IVA}) and Low (L_{IVA}) antibody responder lines that besides their distinct responsiveness to a variety of immunogens, have a clear modification of the macrophage function since the catabolic activity of the cells are higher in the (L_{IVA}) than in the (H_{IVA}) responder lines (Ibañez et al., 1988); InRmax and InRmin mice selected for the acute phase of inflammatory reaction (Ibañez et al., 1992) DBA/2J and A/J mice, both deficient at the C5 Complement protein; Swiss and BALB/c. This *in vivo* assay thus, provides an additional subside on the general activities of the lectin of *D. grandiflora* that actuates as an inflammatory agent.

MATERIALS AND METHODS

Seeds – D. grandiflora seeds were collected in the state of Ceará, Brazil and the lectin was isolated by affinity chromatography on a Sephadex G-50 column (Moreira et al., 1983).

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Animals – The outbred Swiss mice were kept at the Department of Biochemistry and Molecular Biology of the Federal University of Ceará, Brazil. The inbred lines BALB/c, A/J and DBA/2J were kept at the isogenic mouse Animal Room at the Department of Immunology, ICB, São Paulo, Brazil, and Low (L_{IVA}) responder lines of selection IVA, as well as the InRmax and InRmin lines giving maximal or minimal inflammatory responses, respectively, were kept at the Laboratory of Immunogenetics, Instituto Butantan, São Paulo, Brazil. Animals 2-3 month old weighting 20 to 26g were used.

Cutaneous reaction – Subcutaneous injections of 0.1, 0.25, 0.5 or 1.0 mg of the lectin of *D. grandiflora* dissolved, under sterile conditions in 200 µl PBS, were given into the dorsal region of mice. These animals were distributed in groups of five from each line. Control groups received 200 µl PBS in the same conditions. The time course and intensity of the inflammatory reaction were followed by oedema formation (+); extension of the oedema to the roots of mice forelegs (++); beginning of the ulceration of the skin (+++) and ulceration with loosing of necrotic skin (++++). The number of crosses indicates also intensity of the reaction.

Histological analysis – Small fragments of the mouse skin were fixed in formalin, paraffin embedded and stained with hematoxilin and eosin (H/E) or toluidin blue or Giemsa at the 14th day after the injection.

RESULTS

Cutaneous lesion – The cutaneous ulceration was studied at the 14th day after a single injection of 1.0 mg *D. grandiflora* lectin. The histological examination of the ulcerated skin under H/E staining disclosed an exudative reaction predominantly of polymorphonuclear leukocytes with multinucleate giant cells and hemorrhagic areas (Fig. 1). Skin of controls were examined also at the 14th day and they did not exhibit any particular modification (result not shown). The histological analysis performed with toluidin blue and Giemsa methods confirmed those obtained by hematoxilin and eosin method.

Developments of the cutaneous lesion in the several strains of mice – Swiss, BALB/c and InRmax had a similar evolutionary course

Fig. 1: cutaneous lesion induced by subcutaneous injections of 1.0 mg of the lectin of *Dioclea grandiflora* seeds.

TABLE

Time course of the necrotic cutaneous reaction after subcutaneous injection of Dioclea grandiflora lectin

Line	Time course of reaction (days)		
	4	8	14
SWISS	++	+++	++++
BALB/C	++	+++	++++
A/J	+	+	++++
DBA/2J	+	+	++++
HIVA	–	–	++++
LIVA	+	++	++++
InRmax	++	+++	++++
InRmin	–	–	++++

+: presence of loccal oedema.
++: local oedema extended to the roots of forelegs.
+++: presence of skin ulceration.
++++: ulceration with loss of skin.
–: negative result.

of the lesion, i.e. there was a large oedema at the 4th day with ulceration starting at the 8th day (Table). In mice genetically deficient in C5 Complement protein, A/J and DBA/2J, the inflammatory reaction started slowly with mild oedema at the 4th day, increased through the 8th day and the ulceration was fully revealed only at the 14th day. There was a diverse effect between the two lines of mice genetically selected for antibody production. While the L_{IVA} mice started the inflammatory reaction at the 4th day and went on with the ulceration appearing at the 8th day, likely the Swiss, BALB/c and InRmax mice, the H_{IVA} mice did

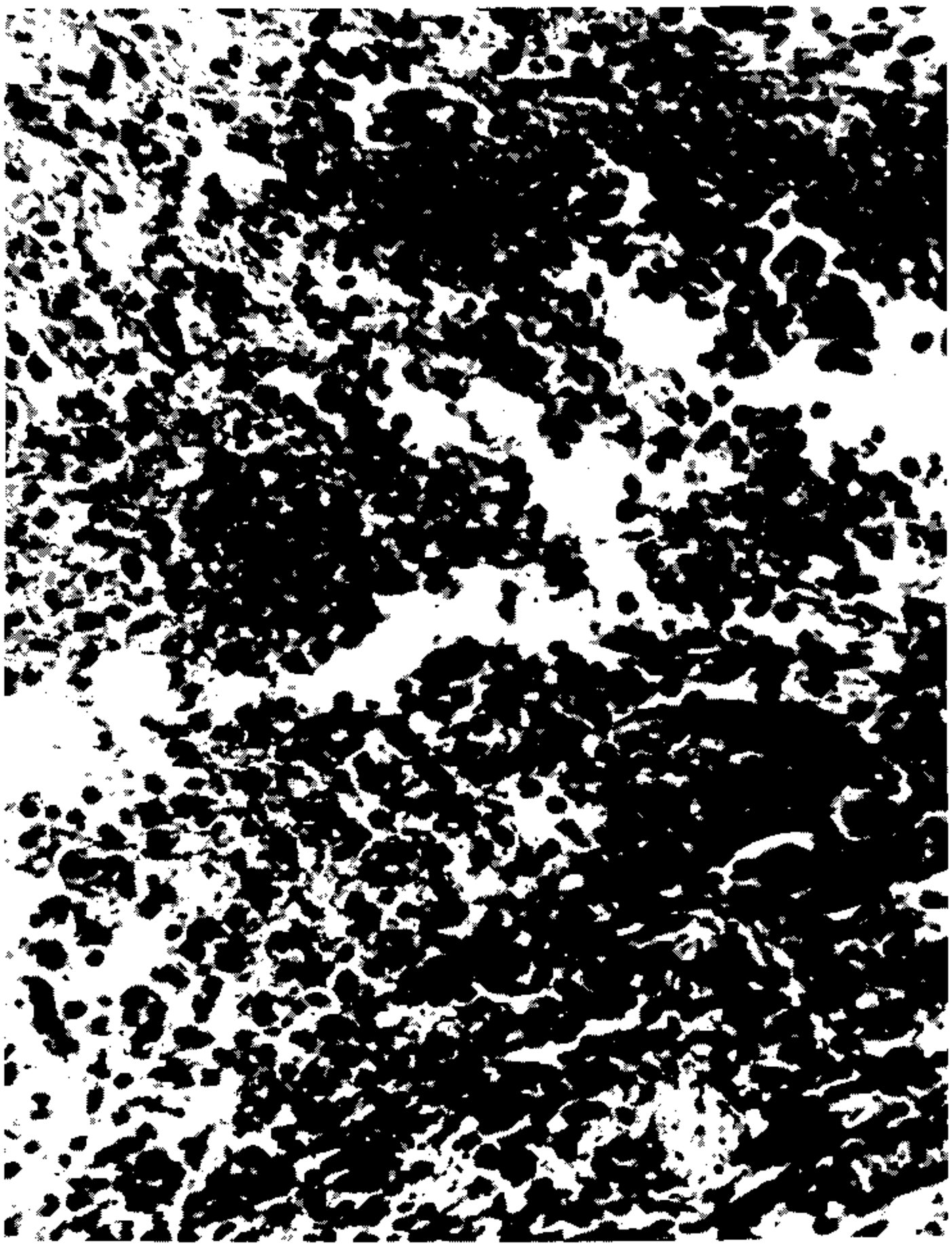

Fig. 2: histological examination of the cutaneous lesion of mice injected with 1.0 mg of the lectin of *Dioclea grandiflora* seeds showing the hemorrhage and the influx of polymorphonuclear leukocytes (H/E staining 100 x magnified).

not exhibit any response neither at the 4th day nor at the 8th day (Table). The features of inflammatory reaction between InRmax and InRmin were also distinct. The first developed a quick reaction with extended oedema through the 8th day, while the InRmin had no response until this time. The C5 deficient inbred A/J and DBA/2J mice developed the same pattern of reaction, showing a slight oedema on days 4 and 8, with a clear ulceration and loss of skin on day 14 (Table).

DISCUSSION

In this work we have studied an inflammatory cutaneous reaction induced by the lectin of *D. grandiflora* in three different strains of mice; the time course of this reaction varied according to the strain of mice. The outbred Swiss, the inbred BALB/c and genetically selected InRmax lines have identical development with an earlier starting of the inflammatory reaction. In the 14th day such reaction was comparable in all strains (Table). This inflammatory process depends on the lectin in its native structure since when heated at 100 °C for 10 hr, it loses hemaglutinatting activity and the capacity to induce such reaction (data not shown). Doses of 0.1, 0.25, 0.5 and 1.0 mg of the lectin induced cutaneous reactions accompanied of skin lesion at different stages according to the dose. It is only presented the time course of the reactions induced by 1.0 mg of *D. grandiflora* lectin because such dose promoted a quicker and clearly defined inflammatory process followed by a net loss of skin than the 0.1, 0.25 and 0.5 mg doses.

The histological analysis of the lesion induced by 1.0 mg *D. grandiflora* lectin revealed by H/E staining method and confirmed by toluidin blue and Giemsa ones showed an inflammatory reaction with an influx of polymorphonuclear leukocytes, presence of giant cells and hemorrhagic areas. In such lesion the presence of lymphocytes and plasma cells was negligible. These data analyzed altogether sugest an inflammatory activity of *D. grandiflora* lectin. Such effect might be exerted through a skin mast cell activation with the release of vasoamine such histamine, cyclooxygenase products, leukotrienes and various enzymes (Siraganian, 1988). This agrees with the fact that the lectin of *D. grandiflora* had already been described to induce exocytosis of mouse peritoneal mast cells (Lima et al., 1985). The participation of mast cells in inflammatory reactions other than immediate hypersensitivity has been frequently demonstrated (Zhang et al., 1992).

As the histological data of the lesion had a certain similarity to the Arthus reaction (Danon, 1980) we have investigated the possible participation of the Complement system in the inflammatory reaction. With the genetically C5 deficient A/J and DBA/2J, *D. grandiflora* lectin induced the same lesion but the time course of it was slower (Table). Apparently, the Complement system plays a role in the mechanism of the skin lesion but it does not seem to be a prominent one.

Employing mice obtained by several selective breeding experiments for antibody response it has been shown that L_{IVA} mice were more susceptible to the inflammatory lesion than the HIVA ones. It is shown that H_{IVA} and L_{IVA} mice respond differently to infection by *Salmonella typhymurium* and this is probably due to differences in catabolic activity of macrophages (Sant'Anna et al., 1989). The enhanced susceptibility to the inflammatory effect of *D. grandiflora* lectin of H_{IVA} mice as compared to L_{IVA} mice might be explained by the differences in catabolic activity of macrophages known to be higher in L_{IVA} mice than in H_{IVA} ones. Thus, L_{IVA} mice could get rid of lectin injected under their skin more quickly, avoiding the inflammatory reaction. The more elevated susceptibility of H_{IVA} mice to the effect of the lectin might yet suggest a poor mobilization of immunological factors. However, further experiments are needed to confirm such hypothesis.

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