male and female specimens respectively indicate that the body weight of the males grew slightly faster than the cube of the length, while in females, this growth in body weight was less than the length cube. This difference in exponent values during the premature phase of the crab implied a sex-related differential in allometry. The fact that the length–weight relationships are considerably influenced by the degree of allometry is evident from the observations of Haefner[2] on the sand shrimp, *Grangon septemspinosa*. Further, the efficiency of the conservation of food into flesh may be higher in the male than in the female.

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## CELL SURFACE DIFFERENCES IN PROMASTIGOTES OF VIRULENT AND AVIRULENT ISOLATES OF *LEISHMANIA DONOVANI*

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SEVERAL parasitic protozoans have been shown to possess cell surface properties responsible for lectin-induced agglutination *in vitro* which correlates with their *in vivo* pathogenicity for the respective hosts[1–4]. The pathogenic strains of *Entamoeba* and *Acanthamoeba* differ from the nonpathogenic strains in their susceptibility to agglutination with concanavalin A[1,2]. The pathogenic trypanosomes have been shown to agglutinate better when incubated with lectins, as compared to nonpathogenic strains. Among the *Leishmania* sp., *Leishmania braziliensis* was found to agglutinate in the presence of concanavalin A and *Ricinus communis* agglutinin[4,5]. In the present communication pathogenicity of three *L. donovani* strains has been characterized using two lectins namely *Ricinus communis* (Sugar specificity: D-galactose; N-acetyl-D-galactosamine) and pea nut (Sugar specificity D-galactose; D-galactose $\beta$-(1-3)-N-acetyl D-galactosamine).

Three different strains of *L. donovani* namely K-15 (isolated from a KA patient of Calcutta obtained from School of Tropical Medicine), $Dd_1$ (isolated from a KA patient of Bihar and obtained from NICD, Delhi), and $Dd_{15}$ (isolated from a KA patient of Bangladesh and obtained from NICD, Delhi), have been used during the present study.

*In vitro* cultures of promastigotes of all the three strains were maintained in NNN medium. To check the *in vivo* pathogenicity of these strains for hamsters, $1 \times 10^7$ promastigotes from each of the cultures were intracardially inoculated into 25 hamsters, each weighing 40 g. Four to five hamsters were sacrificed for each strain on days 15, 30, 60, 120 and 150 post inoculation and the contact smears of the spleen were microscopically observed for the presence of LD bodies. A part of spleen from each hamster was also incubated *in vitro* in NNN medium. These cultures were observed for the presence or absence of promastigotes till day 15.

Two lectins pea nut agglutinin (Sigma) and *Ricinus communis* (Sigma) were used for the agglutination experiment by the method of Dawidowicz *et al*[4] with a slight modification. The serial dilutions of both the lectins PNA and RCA were carried out in microtitre plates, and $2 \times 10^8$ promastigotes of all the three *L. donovani* strains at late log phase (~132 hr) i.e. 5-day-old cultures were incubated (V/V) at 25 ± 1°C for 30–40 min. The agglutination of promastigotes was checked microscopically and was scored from nil (no agglutination) to ++++ (virtually complete agglutination) depending upon the number of the promastigotes/clump and the size of cell aggregate.

On day 15 when hamsters were sacrificed and examined, no LD bodies were observed in the smears of spleen, nor they produced promastigotes in NNN medium with any of the Leishmanial strains. Up to day 150 the hamsters infected with strain $Dd_1$ and $Dd_{15}$ did not show LD bodies in spleen, neither the cultures of spleen were found positive in NNN medium; but in the case of K-15 infected hamsters + infection, ++ infection and ++++ infection in the spleen were observed on days 60, 120 and 150 respectively, and the cultures of such spleens also produced promastigotes in NNN medium (tables 1 and 2).

The agglutination of promastigotes with PNA and RCA showed better agglutination, with strain K-15

**Table 1** *Microscopic examination of spleen smear of hamster*

| *L. donovani* strains | Days | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 120 | 150 |
| K-15 | (−) | (−) | (+) | (++) | (++++) |
| $Dd_1$ | (−) | (−) | (−) | (−) | (−) |
| $Dd_{15}$ | (−) | (−) | (−) | (−) | (−) |

**Table 2** *Microscopic examination of spleen culture in NNN medium*

| *L. donovani* strains | Days | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 120 | 150 |
| K-15 | (−) | (−) | (+) on day 10 | (+) on day 6 | (+) on day 3 |
| $Dd_1$ | (−) | (−) | (−) | (−) | (−) |
| $Dd_{15}$ | (−) | (−) | (−) | (−) | (−) |

promastigotes, when it showed 100% agglutination (++++) at only 10 μg/ml of pea nut, and 240 μg/ml of *Ricinus communis*, but other two strains $Dd_1$ and $Dd_{15}$ showed complete agglutination at 80 μg/ml of pea nut, while the absolute agglutination with RCA up to 480 μg/ml was not observed in the case of these two strains (tables 3 and 4).

The *in vivo* experiments in hamsters and in *in vitro* agglutination with lectins reveal that the pathogenic strain K-15 is better agglutinated in comparison to $Dd_1$ and $Dd_{15}$ (non-pathogenic) strains. This agrees with studies by Ayesta *et al*[5] on the absence of Con A binding receptors and molecules carrying a negative charge on the cell surface of non-pathogenic strain of *L. braziliensis*. Hernandez[6] opined that non-pathogenic promastigotes are probably associated with their low binding and reduced survival compared to pathogenic strains, when invaded on macrophages.

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**Table 3** *Agglutination with pea nut agglutinin (PNA)*

| *L. donovani* strains | Lectin concentration (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 20 | 40 | 80 |
| K-15 | (−) | (+) | (++++) | (++++) | (++++) | (++++) |
| $Dd_1$ | (−) | (+) | (++) | (++) | (+++) | (+++) |
| $Dd_{15}$ | (−) | (+) | (++) | (++) | (+++) | (+++) |

**Table 4** *Agglutination with Ricinus communis agglutinin (RCA)*

| *L. donovani* strains | Lectin concentration (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 120 | 240 | 480 |
| K-15 | (−) | (+) | (++) | (++) | (++++) | (++++) |
| $Dd_1$ | (−) | (+) | (+) | (+) | (++) | (+++) |
| $Dd_{15}$ | (−) | (+) | (+) | (+) | (++) | (+++) |