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EXPRESSION OF CIRCULATING LEUCOCYTES BEFORE, DURING AND AFTER MYIASIS BY *Dermatobia hominis* IN EXPERIMENTALLY INFECTED RATS

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SUMMARY

Expression of circulating white blood cells was investigated in rats (*Rattus norvegicus*) experimentally infected with larvae of *Dermatobia hominis*, the human bot fly. Leucocytes were counted prior to infection (control group) as well as at 6, 10, 15, 20 and 28 days post-infection (dpi) and at 7, 15, 30 and 60 days post-larval emergence (dple). Total leucocyte numbers did not differ markedly among the groups. Significant differences were registered when values from control and animals harboring each larval stage of *D. hominis* were compared; with crescent rank: L_1-, L_2-, control and L_3-infected groups. Leucocyte numbers were significantly higher in the control, 15, 20 or 28 dpi groups than in the 6 dpi animals. Higher counts were observed in control, L_2- or L_3-infected rats than L_1-infected animals. Neutrophils, eosinophils and both large and small lymphocytes were also counted and analyzed. Basophils and monocytes were insufficient in number to permit statistical studies. These results stimulate the continuity of the studies about the host-parasite relationship in the dermatobiosis.

KEYWORD: White blood cells; Myiasis; *Dermatobia hominis*.

INTRODUCTION

The human bot fly *Dermatobia hominis* is the most important Neotropical cuterebrid, its larvae producing myiasis in domestic and wild mammals as well as man. The greatest impact of cutaneous myiasis results from economic losses to meat, milk and hide production[6]. Although infections have been systematically combated, the myiasis remains enzootic in South and Central America[8]. Myiasis caused by *D. hominis* persists for about 40 days in the vertebrate host, causing humoral and cellular changes[15] However little is known about host humoral and cellular responses[9,10,12,15,17,18,19].

The present work aimed to monitor the expression of circulating leucocytes before, during and after experimental myiasis by *D. hominis* and to investigate the relationship between *D. hominis* larvae and their hosts.

MATERIALS AND METHODS

Five groups of five adult male *Rattus norvegicus* (Wistar rats) were infected with four newly hatched larvae of *D. hominis* reared in our laboratory[17]. One sixth group (uninfected animals) was used as control. Smears were made from blood samples taken from the tails of rats from each group: control, at 6, 10, 15, 20 and 28 days post-infection (dpi) and stained with Giemsa. The presence of each larval stage (L_1, L_2 and L_3) associated with host's blood cell was also analyzed. Blood samples were also collected from rats of each group at 7, 15, 30, 60 days post-larval emergence (dple), and from control group (uninfected animals) as well. The white blood cell differential count (156 fields at 100X magnification) was estimated (at least 200 cells/rat) and the results analyzed by Kruskal-Wallis and Mann Whitney U-test using Winstat software. As a rigged control, all rats (not specific pathogen free) were monitored before infections, including by blood smears.

RESULTS

DAYS POST-INFECTION AND CONTROL

Leucocytes and neutrophils : The total number of leucocytes (Table 1) was not significantly different when compared by Kruskal-Wallis test. Significant differences were observed when larval stages (L_1 = 6 dpi, L_2 = 10 + 15 dpi) and L_3 = 20 + 28 dpi) and control animals were compared, with progressively more leucocytes seen in rats infected with L_1, L_2, control and finally L_3. By U-test, leucocytes counts were significantly higher in the control, 15, 20 or 28 dpi groups than in the 6 dpi one. With respect to larval stages, higher counts were seen in control, L_2- or L_3-infected groups than in the L_1-infected group.

Neutrophils were significant, with progressive number in rats at 6, 10, control, 15, 20 and 28 dpi as well as in those infected with L_1, control, L_2 and L_3. Between groups, neutrophils were significantly more numerous in the control *vs* 6 dpi and in 28 dpi *vs* control. With regard

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Table 1
Total number (N), median (M), mean (X) and stand deviation (SD) of neutrophils, eosinophils, small lymphocytes, large lymphocytes and total lymphocyte counted in the blood of rats: control, at days-post-infested (dpi) with *Dermatobia hominis* larvae and days-post-larva emergence (dple)

Group	Neutrophils				Eosinophils				Small lymphocytes				Large lymphocytes				Total lymphocytes			
	N	M	X	SD	N	M	X	SD	N	M	X	SD	N	M	X	SD	N	M	X	SD
Control	217	27.1	25.0	11.8	42	5.2	5.0	4.1	198	24.7	25.0	14.2	532	66.5	66.5	11.3	730	91.2	91.5	25.4
6 dpi	142	17.7	18.0	7.5	32	4.4	3.0	14.9	65	8.1	5.5	9.2	749	93.6	94.5	12.9	814	101.7	100.0	22.1
10 dpi	204	25.5	23.0	11.8	38	4.7	3.5	5.1	152	19.0	14.0	20.3	603	75.4	75.5	17.1	755	94.4	89.5	37.4
15 dpi	300	36.8	38.5	14.5	46	5.7	4.5	6.5	103	12.9	11.0	11.2	548	68.5	68.0	14.3	651	81.3	79.0	25.6
20 dpi	300	37.5	27.0	13.6	43	5.4	5.0	4.9	58	7.2	5.0	7.9	597	74.4	75.0	15.2	655	81.6	80.0	23.1
28 dpi	432	54.0	54.5	15.2	10	1.4	0.0	2.5	61	7.6	4.0	8.3	499	62.4	63.0	15.1	560	70.0	67.0	23.4
7 dple	258	32.2	31.0	11.4	42	5.3	2.5	6.0	188	23.5	23.0	17.1	499	62.4	61.0	13.1	687	85.8	84.0	30.2
15 dple	250	31.2	29.5	11.9	83	10.4	11.0	4.9	106	13.2	11.0	12.7	561	70.1	70.0	15.0	667	83.4	81.0	27.7
30 dple	161	20.1	20.5	10.0	52	6.5	3.5	6.9	122	19.0	14.5	14.8	634	79.2	83.0	19.0	756	98.2	97.5	33.8
60 dple	210	27.5	27.0	11.7	42	5.2	3.5	6.7	41	5.1	2.5	7.1	690	86.2	88.5	18.3	731	91.4	91.0	25.4

to infected groups, significant differences occurred for 10, 15, 20 or 28 dpi *vs* 6 dpi and 28 dpi *vs* 10 or 15 dpi.

Rats with L_3 presented significantly more neutrophils than the control, L_1-or L_2-infected groups. Significant differences were also seen for L_2 *vs* control or L_1 and control *vs* L_1.

Eosinophils and lymphocytes: No significant differences were observed for eosinophils or for total lymphocytes, except for groups: pro- 20 *vs* 6 or 10 dpi.

The only difference with respect to large lymphocytes was seen (between the groups): for 20 *vs* 10 dpi.

Small lymphocytes increased significantly in the ascendant order: 6, 20, 28, 15, 10 dpi and control. Such cells were lowest in rats with L_3 and progressively higher in the L_1, L_2 and control groups. Significant differences (by U-test) were detected in the number of small lymphocytes: pro-control *vs* 6, 15 or 28 dpi and pro-10 or 15 *vs* 28 dpi and pro-control *vs* L_1- or L_3-infected groups.

DAYS POST-INFECTION AND POST-LARVAL EMERGENCE

Leucocytes and neutrophils: No significant differences were found for total leucocytes. Neutrophil numbers increased significantly in the following order of progression: 6 dpi, 30 dple, 10 dpi, 60 dple, control, 15 dple, 15 dpi, 7 dple, 20 dpi and 28 dpi. Neutrophil values were significant for 15 and 20 dpi *vs* 30 dple; 28 dpi *vs* 7, 15, 30 and 60 dple; and 7, 15 and 60 dple *vs* 6 dpi.

Eosinophils and lymphocytes: Eosinophil numbers were progressively higher in groups 28 dpi, 7 dple, 6 dpi, control, 10 dpi, 20 dpi, 60 dple, 30 dple, 15 dpi and 15 dple. Significant differences (between groups) were seen for 15, 30 and 60 dple *vs* 28 dpi.

Total numbers of lymphocytes (small plus large) showed marked variation, increasing progressively as follows: 28 dpi, 20 dpi, 15 dple, 15 dpi, 60 dple, 7 dple, 10 dpi, control, 30 dple and 6 dpi. When two values were compared significant differences were seen for 6 dpi *vs* 7 dple and 15 dple as well as 7, 30 and 60 dple *vs* 28 dpi.

Large lymphocytes increased significantly in the following order of progression: 28 dpi, 7 dple, control, 15 dpi, 20 dpi, 15 dple, 30 dple, 10 dpi, 60 dple and 6 dpi. Significant differences were found between the numbers of large lymphocytes for 6 dpi *vs* 7 and 15 dple, pro-10 dpi *vs* 7 dple; pro-60 dple *vs* 15, 20 and 28 dpi.

Small lymphocyte numbers increased significantly in the following order: 60 dple, 6 dpi, 20 dpi, 28 dpi, 15 dpi, 15 dple, 30 dple, 10 dpi, control and 7 dple. In the comparisons between groups, significant variations of small lymphocyte occurred for 10 dpi *vs* 60 dple and 15 dple *vs* 28 dpi; and pro-7 dple *vs* 6, 20 and 28 dpi.

DAYS POST-LARVAL EMERGENCE AND CONTROL

Leucocytes and neutrophils: No significant differences were found between the total numbers of leucocytes. Significant differences were only seen for neutrophils (U-test): in favour of 7 and 15 *vs* 30 dple.

Eosinophils and lymphocytes: Eosinophils were only significant by U-test: pro-15 *vs* 60 dple.

Lymphocytes are not significant. However, significant differences were seen for large lymphocytes in the following order: 7, control, 15, 30, 60 dple and 60 dple *vs* control. In comparative analyses of the dple periods, significant differences were seen for 15, 30 or 60 dple *vs* 7 and 60 *vs* 15 dple.

The numbers of small lymphocytes were expressive in the following order of progression: 60, 15, 30 dple, control and 7 dple. Such cells lymphocytes showed signification in number only for control *vs* 60 dple and 30 *vs* 60 dple. With regard to dple, significant differences were seen for 7 *vs* 15 or 60 dple and 30 *vs* 60 dple.

DISCUSSION

Cutaneous damage caused by ectoparasites triggers molecular and cellular reactions in the host. For myiasis in particular the larvae-host relationship elicits both non-specific and specific immune responses associated with resident cells and those recruited into inflammatory tissue from blood or lymphatic systems[15]. There have been few previous studies regarding white cells in blood as a result of bot fly infection[7]. Although no significant difference of total leucocytes has occurred among the host groups, the contrasts were observed when the larval stage parasitism are considered, particularly in favour of L_3. These data concur with observations made in cattle infected by *D. hominis*[2]. The reduction in leucocyte numbers during L_1 (6 dpi) and L_2 (10 + 15 dpi) and subsequent increase in L_3 (20 and 28 dpi) may be due to larval chemotactic products inducing haematopoiesis and migration of white blood cells to the inflamed area. This is also consistent with results of studies in rabbits[12] and mice[10] infected by *D. hominis*. The leucocyte number here reported is similar to that observed in cattle infected with *D. hominis*[2] but much lower than that cited for *Tamias striatus* naturally infected by *Cuterebra emasculator*[4]. With respect to the specific types of white blood cells the low numbers of neutrophils at the start of infection, as seen in the present study, may be explained by the fact that such cells are the first to arrive at the site of inflammation[17]. Neutrophilia in the circulation at 28 dpi and during scar formation (7 dple) was much lower than that seen in the skin during inflammation[17]. Expression of eosinophils is more commonly associated with allergic reactions and helminthic parasite infections[1, 13]. Eosinophils are involved in wound healing and repair, in fibrosis, *e.g.* scarring[3]. The constant levels of eosinophlia in the blood during myiasis by *D. hominis* seen during the present study differ from those observed in the skin of several human bot fly hosts in previous studies, including rabbit[11], cattle[15,19] and rat[17,18]. After the L_3 larvae dropped from the host to pupate, the number of circulating eosinophils was greater than during infection, although not significantly compared with the control group. Otherwise eosinophilia has recently been described in sheep infected with the nasal bot fly *Oestrus ovis*[7].

In the present study lymphocytes were similar to those observed in cattle infected with *D. hominis*[2]. Nevertheless, lymphocytosis has been found in bot fly-infected rat skin[16]. Lymphocytopenia observed in cattle with dermal myiasis by *Hypoderma lineatum* were described[5,14].

If compared, the large lymphocyte (lymphoblasts) population remained constant during parasitism by *D. hominis*, but such cells increasing significantly after skin scarring. Nevertheless, small lymphocytes (probably activated cells) fell during myiasis and increased just after the L_3 left the host (at 7 dple).

The prevalence of basophils and monocytes in normal rats[20] were also similar to those found in the present study (insufficient to statistical analysis). In cattle infected with *D. hominis* larvae basophils and monocytes were not expressive[2]. Basophilia occurs in ectoparasitoses such as sheep scab[22] and tick[21].

Knowledge of the variety and numbers of the white cells in host blood during myiasis, through studies such as the one reported here, are essential to focus new research approaches on the relationship between the human bot fly and its hosts.

RESUMO

Expressão de leucócitos na circulação sangüínea antes, durante e após miíase por *Dermatobia hominis* em ratos experimentalmente infectados

A expressão de leucócitos sangüínea foi investigada em ratos (*Rattus norvegicus*) experimentalmente infectados com larvas de *Dermatobia hominis*. As células foram contadas antes, durante, aos 6, 10, 15, 20 e 28 dias pós-infestação (dpi), e aos 7, 15, 30 e 60 dias pós-emergência das larvas dos hospedeiros. O total de leucócitos não apresentou marcante diferença entre todos os grupos de animais. Todavia, diferenças significantes foram observadas quanto ao parasitismo pelos estádios larvares, com nível crescente: L_1, L_2, controle e L_3. Na comparação entre grupos: o número de leucócitos foi significativo pró-controle, -15, -20 ou -28 dpi do que aos 6 dpi; e pró-controle, -L_2 ou -L_3 do que para L_1. Neutrófilos, eosinófilos e linfócitos (pequenos e grandes) foram também analisados. Em contraste, o número insuficiente de basófilos e monócitos não permitiram estudos estatísticos. Estes resultados estimulam a continuação dos estudos sobre a relação parasito-hospedeiro nas dermatobioses.

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