The human lymphocyte cell line, RPMI-1788, does not respond to the in vitro presence of concentrations of cortisol as high as 10^{-5} M (Werthamer and Amaral[6]). Incubation of these cells for 24 h in the presence of purified human transcortin renders these cells responsive to physiological levels of cortisol. The data presented in the table show that a molar concentration of cortisol as low as 10^{-7} inhibits the synthesis of DNA of the lymphocytes that had been incubated n the presence of transcortin, whereas concentrations as high as 10^{-5} have no effect on the synthesis of DNA of unexposed cells. Since the cells of the above experiment were washed prior to exposure to cortisol, the inhibitory effect of cortisol must have involved a change within the cell as the result of exposure to transcortin.

Cortisol sensitized cells as well as the cortisol resistant lymphocytes were homogenized and the cytosols prepared were tested for their ability to bind cortisol. Only the transcortin exposed lymphocytes exhibited a cortisol binding species whose sedimentation behavior in a sucrose gradient was identical to that of transcortin (fig. 1).

Direct application of a fluorescein-labelled transcortin antibody the smears of control (unexposed) and transcortin exposed cells indicated that only the latter contained transcortin (fig. 2).

These results directly support the contention that in the case of human lymphocytes the mobilization of plasma transcortin to the cytoplasmic compartment provides the means by which transcortin may function as a steroid receptor.

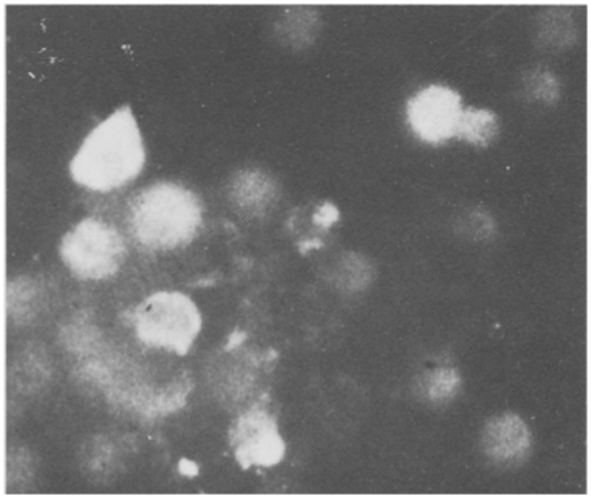

Fig. 2. Demonstration of transcortin within RPMI-1788 lymphocytes exposed for 24 h to purified transcortin. The preparation and application of the fluorescein labelled antibody was previously described[3,4]. (The fluorescein labelled transcortin antibody did not react with unexposed RPMI-1788 cells.)

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Immune T cells control *Trypanosoma cruzi* infections

A.C. Corsini[1] and A. Stelini, Jr[2]

Department of Microbiology and Immunology, University of Campinas, UNICAMP, 13.100 - Campinas (Brazil), 7 July 1980

Summary. Immune but not normal T cells were able fully to restore the ability of thymectomized, irradiated, fetal liver reconstituted 'B' mice to control *Trypanosoma cruzi* infections.

Several effector mechanisms are triggered by the host to control parasites[3]. Thus, in *Trypanosoma cruzi* infections antibodies of the IgG_{2a} and IgG_{2b} subclasses, but not the IgM class, were demonstrated to be protective[4]. Complement was also important in natural resistance[5,6].

In addition, it has been demonstrated that lymphocytes harvested from the spleen and lymph nodes of infected animals decreased parasitaemia and mortality in newly infected syngeneic recipients[7-9]. Moreover, 'nude' mice grafted with thymus from syngeneic donors were resistant to the infection in contrast with non-grafted controls[10]. Thus, a T lymphocyte-mediated effector mechanism has been suggested to regulate the outcome of the infection with *T. cruzi*[9].

In this paper we present evidence that immune but not normal T cells are necessary to control *T. cruzi* infections in thymectomized, irradiated, fetal liver reconstituted mice. In addition, protection was not achieved in recipients of immune serum.

Material and methods. (CBA × C57 BL/10) F_1 mice bred and kept in our animal facilities were 3-4 months old when infected. *Trypanosoma (Schizotrypanum) cruzi* strain Y, passaged weekly in Swiss 55 mice[11] was used to infect mice. Mice were infected i.p. with 100 parasites as described previously[12].

Thymectomized, irradiated, fetal liver reconstituted mice (B mice) were prepared following instructions[13]. The thymuses were surgically removed by opening the sternum of 1-month-old mice. 1 month later the animals were irradiated (850 rad-Co^{60} source) and immediately reconstituted with 5×10^6 fetal liver cells from 12-15-day-old syngeneic embryos.

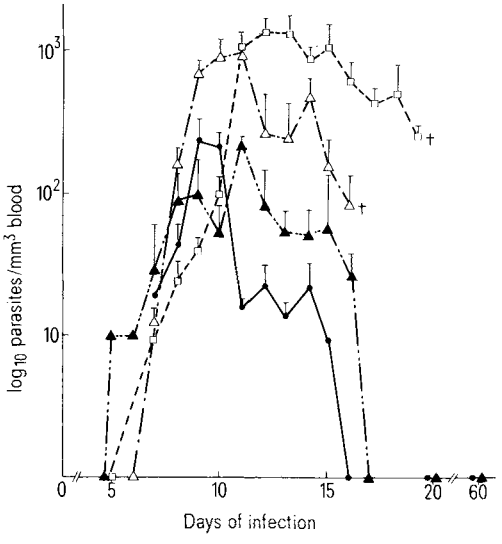
Nylon wool purified T cells were prepared as described previously[14] with minor modifications. Briefly, 2×10^8 spleen cells harvested in Eagle-Minimum Essential Medium (Eagle-MEM) containing 1% of fetal calf serum (FCS) from either normal or 30-day infected mice (immune cells) were resuspended in 40 ml of 0.85% NH_4Cl for

5 min to remove red blood cells then washed 2× in Hanks balanced salt solution (HBSS). The cells were resuspended in 1 ml phosphate buffered saline (PBS)+5% FCS and loaded over a 5 ml syringe containing 0.6 g of nylon wool (Rhodia-3.3 dtex SO) previously soaked with PBS-5% FCS and kept at 37 °C. The cells were harvested after 30 min and passed through a 2nd column under the same conditions.

The cells were washed in HBSS, resuspended in Eagle-MEM-1% FCS and then i.v. injected into mice. This cell population containing less than 5% surface Ig bearing cells as determined by immunofluorescence, was considered as purified T cells.

Immune sera from chronically infected mice (minimum 30-day period post-infection) were pooled, inactivated (56 °C, 30 min) and kept at −20 °C. Mice were injected i.v. every other day with 0.2 ml. The parasitaemia was followed-up daily in every single mouse as described previously[11].

Results. The figure shows the mean parasitaemia in groups of mice infected with *T. cruzi*. Thus, normal irradiated



Parasitaemia in T-deprived (CBA×C57 BL/10)F_1 mice infected with *T. cruzi*. Mice infected i.p. with 100 parasites. Each point represents the geometric mean±SD (log 10) of 8–10 mice/day. ●——●, Normal, irradiated, fetal liver reconstituted. □----□, Thymectomized, irradiated, fetal liver reconstituted (B) mice. △-·-△, B mice+normal T cells. ▲-·-▲, B mice+immune T cells.

Mortality in T-deprived (CBA×C57 BL/10)F_1 mice infected with *T. cruzi*

	Mortality 20 days	30 days
Normal	0/10	0/10
Normal, ♀, FL reconstituted	0/10	1/10
B mice	13/13	-
B mice + immune sera	10/10	-
B mice + normal T cells (2×10^7)	13/13	-
B mice + immune T cells (10^7)	4/10	6/10
B mice + immune T cells (2×10^7)	1/10	1/10

Mice infected with 100 parasites. B mice = thymectomized, irradiated (♀, 850 rad) and fetal liver (FL) reconstituted. Immune cells = spleen cells from chronically infected normal mice. Numerator indicates number of dead mice, denominator number of infected mice.

reconstituted mice developed an increasing parasitaemia from the 5th day after infection peaking on day 9 (300 parasites/mm^3). Mice usually controlled parasitaemia from the 15th day onwards keeping very low numbers of circulating parasites undetected by the visual method. This pattern was quite similar to infections in normal F_1 mice[15]. On the other hand, B mice developed an uncontrolled parasitaemia, peaking on day 11 (1350 parasites/mm^3). Although circulating parasites decreased by day 15, parasitaemia was still very high around day 18. No mice survived longer than 20 days. B mice were not protected with 0.2 ml of immune sera given i.v. every other day (table), although the mean parasitaemia was lower (not shown). B mice reconstituted with normal T cells (2 and 4×10^7 cells) 1 month previously to infection were also extremely susceptible to the infection although mice peaked with lower parasites numbers (950 parasites/mm^3) compared to unreconstituted mice. Parasitaemia decreased around day 12 but no mice survived longer than 16 days of infection (table).

Time between reconstitution with normal T cells and infection was increased up to 2 months, but did not change the outcome of the infection. Higher numbers of normal T cells were not tested.

However, B mice reconstituted with immune T cells (2×10^7) 1 month previously not only survived infection but developed also the lowest mean parasitaemia (lower than normal controls: 200 parasites/mm^3 at the peak). Mortality was 10% in this group. Higher numbers of immune T cells (4×10^7) neither changed the pattern of the infection nor decreased mortality.

Discussion. Our results confirm the previous reports describing the essential role played by the thymus derived lymphocytes in controlling the outcome of *T. cruzi* infections in mice[10,16]. However, differently from Burgess and Hanson's results[9] where a variable degree of protection could be transferred with normal spleen cells our B mice reconstituted with normal T cells were unable to cope with the infection. Since maturation of those transferred cells was likely to be important, we increased the time between transfer and infection up to 2 months without any benefit. On the other hand, immune T cells were extremely efficient in controlling parasitaemia and mortality in B mice independently on the time between reconstitution and infection. Those T-immune reconstituted mice performed better than normal mice as far as the parasitaemia was concerned. It is important to stress that T cells harvested from the spleen of mice immunized with live epimastigotes were also very effective in transferring protection to syngeneic C57 BL/10 recipients challenged with trypomastigotes in contrast with less efficient B cells[17].

The data suggest that besides their co-operation in antibody production T cells may participate in cytotoxic reactions against the invading trypanosome as suggested previously[9,10]. In addition, macrophages[18] and eosinophils[19] were demonstrated to be cytotoxic to *T. cruzi*.

Macrophages were demonstrated to be activated by immune T cells in vitro and to kill their intracellular amastigotes[20]. Although it is difficult to correlate data obtained from in vitro experiments with those obtained in vivo it is possible that such a reaction was also effective in our experimental model.

If this is true, we suggest that humoral and cell-mediated reactions may act in different phases of the infection. Thus, cell-mediated reactions at the beginning of the infection would decrease the infective dose of parasites till an effective humoral response could be mounted. Experiments are being carried out in normal T cells reconstituted B mice treated with immune sera to test this hypothesis.

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The negative correlation between erythrocyte concentration and mean corpuscular haemoglobin or mean corpuscular volume in normal dogs

J. Berger

Laboratory of Haematology, Research Institute for Pharmacy and Biochemistry, CS-53351 Pardubice-Rosice (Czechoslovakia), 15 August 1980

Summary. In normal adult Beagle dogs red cell concentration decreased linearly with increasing mean red cell volume and mean red cell haemoglobin. The results are statistically significant ($p < 0.001$).

In toxicological studies performed on Beagle dogs we have observed, in intact animals, negative coefficients of correlation between red blood cell counts (RBCC) and mean corpuscular haemoglobin (MCH) or between RBCC and mean corpuscular volume (MCV). We have found no report on such a relationship of RBCC and MCH or MCV of dogs in the literature. The observed relationships have a bearing on studies of erythropoiesis, clinical interpretation of laboratory data etc., as laboratory dogs are frequently used as a model in biological and medical experiments.

Material and methods. Adult Beagle dogs at the age of 9–49 months (201 males and 197 females) were studied. They were kept individually in air-conditioned indoor dog cages, 0.9×1.0 m. A dry dog diet in pellet form without supplemental vitamins, was supplied at the rate of 0.33 kg/day per animal; water was available ad libitum. The lights were automatically controlled on a 12-h on, 12-h off cycle. Blood samples were taken from the vena cephalica antebrachii 1–3 h after the lights were switched on. Each dog was examined once only. Erythrocyte counts were determined electronically on Celloscope 101 and Picoscale PS-4 counters, haemoglobin on Linson Photometer (540 nm), haematocrit by a micromethod in capillary tubes[1]. All devices were calibrated. MCV was computed from haematocrit and RBCC, MCH from haemoglobin level and RBCC (using Hewlett Packard 1000/40 computer). A least-squares fit was used to determine the slope and intercept for a linear relation[2].

Results. The results are shown in the table. Negative values of correlation coefficients between RBCC and MCH or MCV were obtained. These coefficients of correlation were significant ($p < 0.001$). No significant differences between correlation coefficients for males and females were revealed ($p = 0.38$ for $r_{MCH,RBCC}$, $p = 0.41$ for $r_{MCV,RBCC}$). Therefore, data obtained in males and females were subsequently analyzed together and the results of linear regression analysis are shown in the figure. 2 regression lines are in each part of the figure. One shows the dependence of RBCC on MCH or on MCV (RBCC is on the left-hand side of the equations), the other shows the dependence of MCH or MCV on RBCC (MCH and MCV are on the left-hand side in the equations). As values of correlation coefficients between RBCC and MCH or MCV are similar, the statistical significance of the correlation difference between RBCC and MCV or MCH respectively is for $p = 0.024$; we also computed the coefficient of correlation between MCV and MCH (table); MCV correlates with MCH.

At the same time we computed variation coefficients for the characteristics concerned in males (V_M) and females (V_F) and we found a lower constancy of MCV ($V_M = 14.0$, $V_F = 14.2\%$), MCH ($V_M = 15.1$, $V_F = 14.4\%$) and RBCC ($V_M = 14.1$, $V_F = 15.7\%$) than MCHC ($V_M = 9.8$, $V_F = 7.4\%$),

Coefficients of correlation (r) between values of red blood cell concentration (RBCC) and mean corpuscular haemoglobin (MCH) or between RBCC and mean corpuscular volume (MCV)

Characteristic	Males	Females	Males and females
RBCC ($10^{12}/l$)	7.03 ± 0.07[a]	7.12 ± 0.08	7.07 ± 0.05
MCH (pg)	24.53 ± 0.26	24.25 ± 0.25	24.39 ± 0.18
MCV (fl)	75.88 ± 0.75	75.23 ± 0.76	75.56 ± 0.53
$r_{MCH,RBCC}$	−0.741	−0.793	−0.767
$p(r_{MCH,RBCC})$	<0.001	<0.001	<0.001
$r_{MCV,RBCC}$	−0.806	−0.845	−0.826
$p(r_{MCV,RBCC})$	<0.001	<0.001	<0.001
$r_{MCV,MCH}$			+0.791
$p(r_{MCV,MCH})$			<0.001
number of animals	201	197	398

[a] Means ± SEM. $p(r_{MCH,RBCC})$, $p(r_{MCV,RBCC})$ and $p(r_{MCV,MCH})$ are levels of statistical significance of the correlation coefficients concerned.