might be possible as another method useful for the detection of cellular immunity to defined antigens, although so far its usefulness is limited to PPD.

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Cortisol resistant RPMI-1788 lymphocytes become sensitive to cortisol subsequent to a 24-h incubation period in medium containing purified human transcortin[1]

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Summary. RPMI-1788 lymphocytes (a human cell line) are resistant to cortisol in vitro. Prior incubation for a minimum of 24 h in a medium which contains purified human transcortin at a concentration of 50 µg/ml renders these cells sensitive to the inhibitory action of cortisol as regards the synthesis of DNA. Only the transcortin-exposed cells contain a cortisol binding species whose sedimentation behavior in a sucrose gradient is identical to that of transcortin.

The response of a target cell to a steroid is predicated upon the existence of a steroid-specific, cytoplasmic receptor protein that is physiologically active[2]. Some cortisol responsive cells of man contain cortisol-binding proteins that exhibit physio-chemical and immunological characteristics that are similar to human transcortin[3-5]. The role of this transcortin-like protein has been postulated to be that of a receptor[3].

RPMI-1788 lymphocytes continuously cultured in our laboratory were washed 3 times in serum-free, TC199 medium each wash being followed by centrifugation at $850 \times g$ for 20 min at 4 °C. The pellet of cells was suspended in cold medium and the concentration of cells adjusted to 5×10^5/ml. To duplicate flasks containing 25 ml of the cell suspension, heat-denatured fetal calf serum was added to make a final concentration of 15%. To one of the flasks, lyophilized purified transcortin[3] was added to yield a final concentration of 50 µg/ml. The flasks were incubated for 24 h, the cultures were centrifuged and the cell pellets washed 3 times with ice cold TC 199 medium. The last pellets were suspended in ice cold TC 199 medium to yield a cell count of 5×10^5/ml. Aliquots of 1 ml from each flask were transferred to separate triplicate tubes containing 50 µl of the vehicle used for the preparation of cortisol[7]. To each culture 1 µCi of ^{3}H-thymidine (New England Nuclear Corp.) sp. act. of 22.4 Ci/mm was added and the cultures incubated for 2 h at 37 °C. The cpm of radioactive thymidine incorporated by each culture were obtained by methods previously described[5]. The percent deviation from the mean cpm of the triplicate set did not exceed 5%.

The effect of cortisol on the incorporation of ^{3}H-thymidine by RPMI-1788 lymphocytes cultured in the absence and presence of purified human transcortin

Prior 24 h incubation with 15% fetal calf serum	cpm of ^{3}H-thymidine/10^5 cells ± SD	Difference
Control	129,590 ± 835	-
Cortisol 10^{-8} M	131,250 ± 1040	+2%
Cortisol 10^{-7} M	130,400 ± 880	0%
Cortisol 10^{-6} M	130,280 ± 650	0%
Cortisol 10^{-5} M	129,890 ± 810	0%
Prior 24 h incubation with 50 µg of transcortin/ml of medium and 15% calf serum	**Average cpm of ^{3}H-thymidine/10^5 cells ± SD**	**Difference**
Control	97,440 ± 935	-
Cortisol 10^{-8} M	96,050 ± 1090	−2%
Cortisol 10^{-7} M	72,910 ± 625	−25%
Cortisol 10^{-6} M	59,630 ± 710	−39%
Cortisol 10^{-5} M	41,380 ± 470	−58%

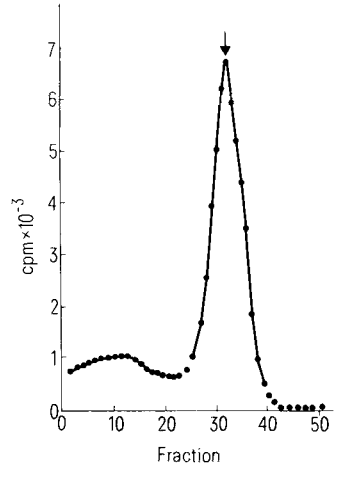

Fig. 1. Sucrose gradient sedimentation of cortisol binding species of cytosols obtained from cortisol-responsive (transcortin exposed) an cortisol-unresponsive RPMI-1788 lymphocytes. Cytosols were prepared from 10^8 transcortin-exposed and unexposed RPMI-1788 cells by procedures previously described[2]. Aliquots of 1 ml containing approximately 1 mg of protein were incubated with 10^6 cpm of ^{3}H-cortisol for 1 h at 4 °C. At the end of this period, 1 mg of dextran-coated charcoal was added to each tube, the tubes incubated for 1 h in a shaking water bath maintained at 4 °C and then centrifuged at $6000 \times g$ for 20 min. Duplicate aliquots of 0.3 ml were layered on top of 5-20% sucrose gradients and centrifuged for 21 h at 4 °C at 60,000 rpm in a Beckman SW-65 rotor. The gradients were fractionated as previously described[3]. (The arrow denotes the fraction coresponding to the peak cpm of cortisol bound by purified transcortin.)

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.

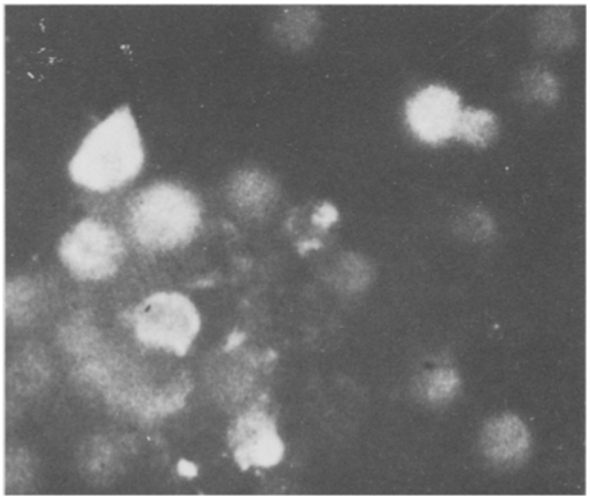

Fig. 2. Demonstration of transcortin within RPMI-1788 lymphocytes exposed for 24 h to purified transcortin. The preparation and application of the fluorescein labelled antibody was previously described[3,4]. (The fluorescein labelled transcortin antibody did not react with unexposed RPMI-1788 cells.)

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Immune T cells control *Trypanosoma cruzi* infections

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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