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Testosterone-induced Conversion from Non-lethal to Lethal *Plasmodium chabaudi* Malaria is not Associated with Changes in Protein Synthesis of Intraerythrocytic Parasites

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Testosterone (T) is known to increase the susceptibility towards a wide variety of infectious diseases (Alexander and Stimson 1988). Recently, T has been found to dramatically affect the outcome of infections with the murine malaria parasites *Plasmodium chabaudi* (Wunderlich et al. 1988) and *P. berghei* (Kamis and Ibrahim 1989). For instance, *P. chabaudi* infections take a self-healing course at low circulating T-levels, as in female and male castrated B10 mice, whereas they are lethal at high T-levels, as in intact male B10 mice or T-treated female and castrated male B10 mice (Wunderlich et al. 1988; 1991). The mechanisms by which T converts non-lethal to lethal *P. chabaudi* malaria are not yet thoroughly understood. One possibility not examined to date is that T directly causes the parasites to change from a non-lethal to a lethal phenotype. If so, T is expected to affect protein synthesis of parasites, since T normally mediates its effects through genomic mechanisms. It is well established that T bound to androgen receptor activates specific genes and, thus, the expression of cell-specific mRNA and proteins, respectively (Norman and Litwack 1987). Indeed, there is information available that T, as other steroids too, can directly act on parasites and that several parasites contain sex steroid binding proteins (Kiser et al. 1986; Lawrence 1991). The results described in this paper, however, demonstrate that the T-induced conversion from non-lethal to lethal *P. chabaudi* malaria is not associated with any significant changes in protein synthesis of intraerythrocytic parasites and that the latter do not contain any specific T-binding proteins.

Blood stage infections of *P. chabaudi* normally take a self-healing course in female B10 mice (Fig. 1). However, a lethal outcome is observed when the mice are treated with T. This T-induced conversion from non-lethal to lethal *P. chabaudi* malaria is associated with changes in parasitaemia. Indeed, *P. chabaudi*-infected erythrocytes are more frequent in the peripheral blood on day 4-8 p.i., and the peak parasitaemia is about 10-15 % higher and occurs approximately 1 day earlier in T-treated than in untreated mice (Fig. 1).

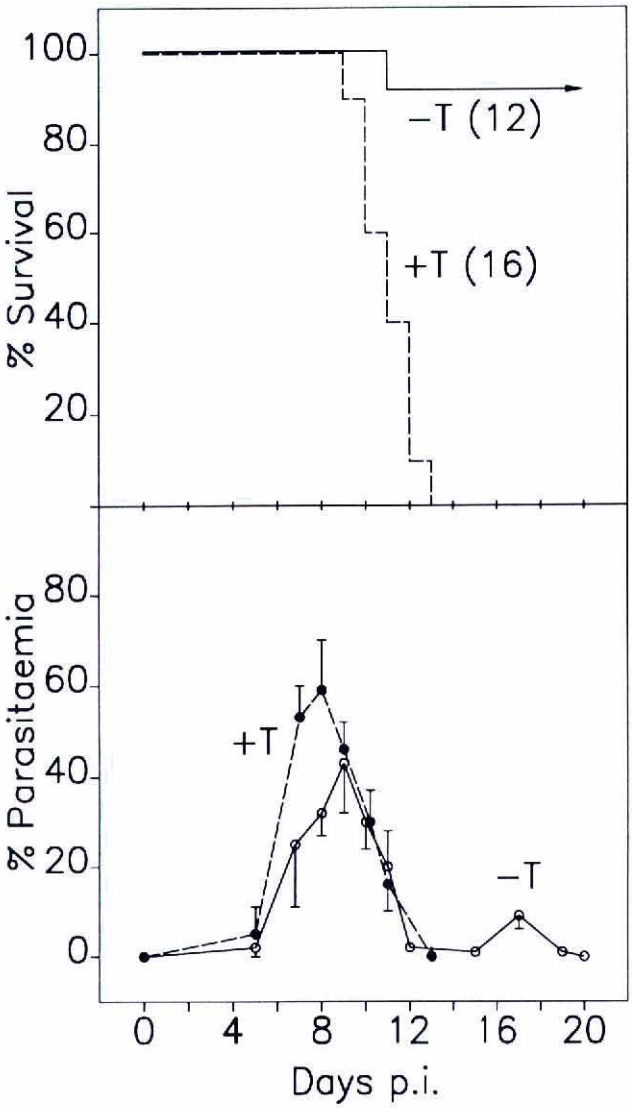

Fig. 1. Effect of T on *P. chabaudi* infections in female C57BL/10 mice. Mice, 8-10 weeks old, were subcutaneously treated with 0.9 mg T (Testosterone Depot 50, Schering, Berlin) in 200 µl sesame oil, twice a week, for 3 weeks (Benten et al. 1991). Controls (-T) received sesame oil alone. Mice were then challenged with 10^6 *P. chabaudi*-infected erythrocytes and the T-treatment was continued during infections. Parasitaemia was examined in Giemsas-stained blood smears. Numbers in brackets indicate total number of mice. Bars indicate SD.

P. chabaudi-infected erythrocytes isolated at a parasitaemia of 20-35 % reveal about 60 major and many minor spots in silver-stained 2D gelelectrophoretograms. The molecular masses of these proteins range from about 8 kDa to about 150 kDa and the isoelectric points from about 7.5 to about 4.0. Though some variations always occur in the appearance of some spots from one experiment to another, the protein pattern is not significantly different between parasites isolated from untreated and T-treated mice. Metabolic labeling with ^{35}S-methionine of *P. chabaudi*-infected erythrocytes and subsequent protein separation by isoelectric focusing/sodium dodecyle sulfate gel electrophoresis (IEF/SDS-PAGE) does not reveal any significant differences in protein synthesis of parasites from untreated and T-treated mice (Fig. 2), even if the metabolic labelling of *P. chabaudi*-infected erythrocytes from T-treated mice is performed in the presence of 100 ng T/ml. Moreover, mRNA expression of intraerythrocytic parasites is not affected by T as examined by *in vitro* translation (Fig. 2).

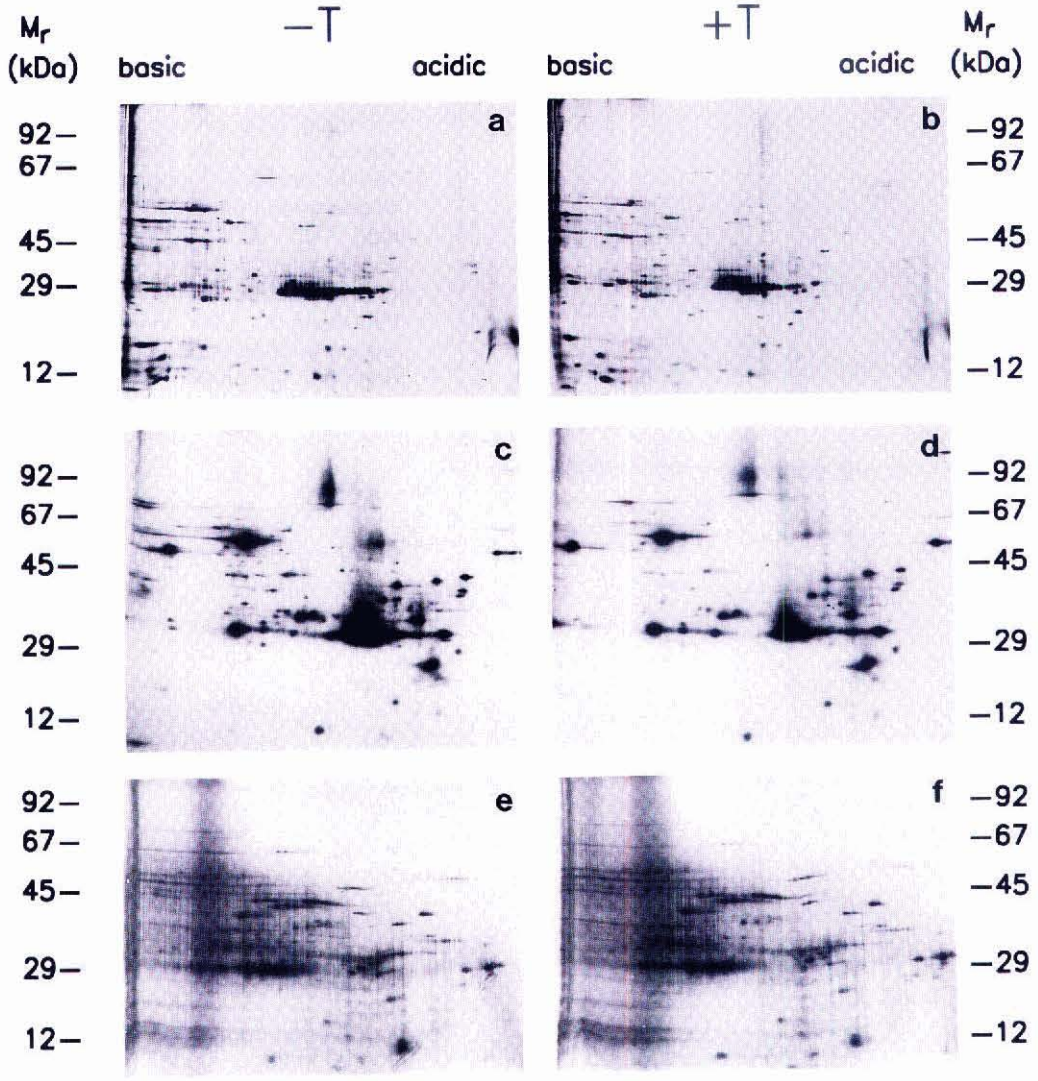

Fig. 2. 2D profiles of *P. chabaudi* proteins. B10 mice were treated with T and challenged as described in legend to Fig. 1.

a, b) *P. chabaudi*-infected erythrocytes were prepared as detailed earlier (Wunderlich et al. 1987). After removal of hemoglobin from *P. chabaudi*-infected erythrocytes by lysis in 0.01 % saponine, proteins were subjected to IEF/SDS-PAGE (Schmitt-Wrede et al. 1991) and silver stained. a) control, b) from T-treated mice.

c, d) *P. chabaudi*-infected erythrocytes (2.5 x 10^8/ml) were incubated with 300 μCi L-[^{35}S]-methionine (spec. act.: > 800 Ci/mmol; Amersham, Braunschweig, F.R.G.) in 3 ml methionine-depleted RPMI-medium (Biochrom, Berlin, F.R.G.) supplemented with 10 % FCS (Boehringer, Mannheim, F.R.G.) and 2 g/l $NaHCO_3$ for 1 h at 37 °C under 6 % CO_2. Protein separation by IEF/SDS-PAGE and fluorography was done as described previously (Schmitt-Wrede et al. 1991). c) control, d) from T-treated mice.

e, f) *In vitro* translation was performed with 5 μg total RNA isolated from *P. chabaudi*-infected erythrocytes using ^{35}S-methionine and a reticulocyte kit (type I, Boehringer, Mannheim, F.R.G). Proteins were separated by IEF/SDS-PAGE and visualized by fluorography. e) control, f) from T-treated mice.

Finally, we have examined parasites for the presence of T-binding proteins using the dextran-coated charcoal method (McGuire 1970), with the synthetic androgen ^{3}H-methyltrienolone as radioligand (Table 1). No T-binding proteins could be detected in the 105,000xg supernatant at concentration ranges of the radioligand of 10 nM - 75 nM, though the sensitivity of this T-binding assay is in the fmol range (Mc Guire 1975).

Table1: Binding of ^{3}H-methyltrienolone to *Plasmodium chabaudi*

Sample No.	^{3}H-methyltrienolone, final conc. (M)	methyltrienolone, final conc. (M)	dpm bound/200 µl
1	$1x10^{-8}$	---	13710
1'	$1x10^{-8}$	$1x10^{-5}$	13144
2	$1.5x10^{-8}$	---	21032
2'	$1.5x10^{-8}$	$1x10^{-5}$	20720
3	$7.5x10^{-8}$	---	28989
3'	$7.5x10^{-8}$	$1x10^{-5}$	29679

Parasites were freed from host erythrocytes and purified on percoll gradients as described in detail previously (Wunderlich et al. 1987). The samples of *P. chabaudi* were homogenized with sonification in 2 volumes of homogenization buffer (0.25 M sucrose, 10 % glycerol, 1 mM EDTA, 0.25 mM DTT, 0.5 mM PMSF, 0.1 mM antipain, 0.1 mM leupeptin and 10 mM Tris-HCl, pH 7.5) at 4 °C. The homogenate was centrifuged at 105,000xg for 90 min. Clear supernatant was used for receptor determination. Protein concentration was estimated by the method of Lowry et al. (1951) and was 2 mg/ml. The steroid binding was estimated by the modified method described by McGuire (1975). Supernatant was incubated with increasing amounts (three points) of labeled ^{3}H-methyltrienolone (85.7 Ci/mM) ranging from $1x10^{-8}$ to $7.5x10^{-8}$ M in the absence and presence of 500 fold excess of unlabeled methyltrienolone. The unbound ligand was removed by charcoal-dextran.

Our data seem to indicate that the T-induced conversion from non-lethal to lethal *P. chabaudi* malaria in female B10 mice is associated with an apparent increase in virulence of parasites. *A priori*, this apparent increase in infectious virulence of parasites could be either due to a direct effect of T on the parasites or reflects T-effects on the hosts. According to the mainstream view, T mediates its effects through an androgen receptor (Norman and Litwack 1987). Remarkably, however, we have been unable to detect any T-binding protein in the intraerythrocytic parasites, and it is therefore not surprising that T does also not affect the pattern and synthesis of parasitic proteins detectable by the methods used. Even if *P. chabaudi* parasites were more virulent in mice under direct T-influence, it appears more likely that T exerts its effect on parasites mainly -if not exclusively- through the host. Such indirect host-mediated T-effects on the parasites might be rather complex. The extreme alternatives are that T promotes host factors increasing parasite virulence in mice or that T does not affect parasitic virulence at all. In support of the latter view, T is known for its immunosuppressive activity (Grossman 1989). In particular, T has been shown to suppress the formation of protective IgG-antibodies in B10 mice (Wunderlich et al. 1992). In addition to the immunosuppressive T-effects, T may also disturb other

events in hosts ultimately contributing to an apparent more virulent course of *P. chabaudi* infections. Thus, T could decrease splenic clearance or the endogenous sequestration of parasitized erythrocytes and/or could change the properties of erythrocytes facilitating parasite invasion.

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