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# Novel Gene Expressed in Spleen Cells Mediating Acquired Testosterone-Resistant Immunity to *Plasmodium chabaudi* Malaria

Jürgen Krücken, Hans-Peter Schmitt-Wrede, Ulrich Markmann-Mulisch, and Frank Wunderlich[1]
*Division of Molecular Parasitology and Biological–Medical Research Centre, Heinrich-Heine-University, 40225 Duesseldorf, Germany*

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**We report the identification of a novel mouse cDNA encoding IAP38, a putative plasma membrane protein of 38 kDa in splenic macrophages, B cells and T cells. The expression of *iap38* is induced by blood-stage infections of *Plasmodium chabaudi* malaria and is testosterone-sensitive in non-immune mice. However, when mice have acquired testosterone-resistant immunity to *P. chabaudi,* there is an about 40-fold increase in the expression of *iap38,* which has then largely lost its responsiveness to infection and testosterone. The gene *iap38* is suggested to be involved in imparting spleen cells the ability to mediate testosterone-resistant immunity to *P. chabaudi* malaria.** © 1997 Academic Press

Though still largely disregarded, there is considerable evidence that the endocrinium is critically involved in the control of immunity and outcome of numerous infectious diseases (1–3). The murine malaria *Plasmodium chabaudi* is an appropriate model to study, for example, the effect of testosterone on immunity and outcome of infections. C57BL/10 mice are able to self-heal blood-stage infections of *P. chabaudi* and, thus, acquire long-lasting immunity to homologous rechallenge (4). However, testosterone suppresses the development of protective immunity which manifests itself as a fatal outcome of infections (5,6). Remarkably, however, there occurs a switch from testosterone-sensitivity to testosterone-resistance with progressing establishment of immunity-mediating mechanisms. Indeed, when once existing, protective immunity is not responsive to testosterone at all (7). Obviously, testosterone-resistance is a peculiar inherent feature of acquired immunity to *P. chabaudi* malaria.

The spleen is one of the major lymphoid organs in the defense against blood-stage malaria (8). In C57BL/10 mice, spleen cells have been shown to mediate both testosterone-inducible immunosuppression and testosterone-resistant acquired immunity to *P. chabaudi* malaria. Thus, testosterone induces spleen cells of non-immune mice to change to an immunosuppression-mediating phenotype (9). In contrast, acquisition of protective immunity coincides with a change of spleen cells to a testosterone-resistant phenotype (7). Here, we provide evidence that the acquisition of the testosterone-resistant phenotype of spleen cells is associated with the abundant expression of a novel gene encoding a putative plasma membrane protein.

## MATERIALS AND METHODS

*Mice and infections.* Mice bred under specific pathogen-free conditions were obtained from our animal facilities. They were housed in plastic cages and received standard diet and water *ad libitum*. Blood-stage infections of *Plasmodium chabaudi* were routinely maintained in NMRI-mice by weekly passages of infected blood (10). Parasitaemia was examined in Giemsa's-stained smears of tail blood. Erythrocytes were counted in a Neubauer chamber. All experiments were performed with female mice of the inbred strain C57BL/10. Mice were made immune by vaccinating at an age of 8–12 weeks with $10^6$ erythrocyte ghosts isolated from *P. chabaudi*-parasitized erythrocytes of NMRI-mice before challenging with $10^6$ *P. chabaudi*-infected erythrocytes as detailed previously (11). All mice cleared fulminant parasitaemias within two weeks. Parasitized erythrocytes had completely disappeared from peripheral blood on week 3 *post infectionem*. Mice on week 9 *post infectionem* were used as immune mice throughout the experiments.

*Testosterone-treatment.* Immune mice and non-immune control C57BL/10 mice of the same age were subcutaneously injected with 0.9 mg testosterone in 100 $\mu$l sesame oil or sesame oil alone twice per week for 3 weeks as detailed previously (12). In some experiments, testosterone-treated immune and non-immune mice as well as corresponding mice not treated with testosterone were infected with $10^6$ *P. chabaudi*-infected erythrocytes for 7 days. Experimentation was

[1] Corresponding author. Fax: +49 211 81 14734.

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approved by state authorities and followed the German law on animal protection.

*Isolation of spleen cell populations.* Spleens were aseptically removed, passed through a stainless steel sieve, and depleted of erythrocytes by $NH_4Cl$ lysis as described recently (9). Adherent cells as a source of macrophages were prepared by incubating total nucleated spleen cells in plastic petri dishes at 37°C for 2 hrs. The non-adherent cells were removed and separated in T cells and B cells by magnetic cell sorting (13). For this, cells were labeled with biotin-conjugated anti-Ig monoclonal antibody, streptavidin-FITC and biotin-conjugated magnetic microparticles and passed though a magnetized column. The non-bound cell fraction contained T cells as $Ig^-$ cells, whereas B cells were derived by elution of the bound fraction.

*Isolation of RNA.* Total RNA was extracted according to the GTC/CsCl cushion method as described previously (14) or by a single step acid GTC/phenol/chloroform extraction (15). Poly(A)$^+$ RNA was isolated by oligo(dT) cellulose chromatography (16).

*Differential screening of a cDNA-library.* A cDNA-library was constructed from a spleen cell fraction pooled from immune mice according to the Stratagene cDNA kit protocol (Stratagene, Heidelberg, Germany). The cDNA was ligated into EcoRI/XhoI cleaved Uni-ZAPXR bacteriophage vector (Stratagene) and packaged *in vitro* (Gigapack II Gold, Stratagene). The cDNA-library consisted of approximately $1.5 \times 10^6$ non-amplified recombinant phages. Differential screening using $^{32}$P-labeled cDNA synthesized from RNA of spleen cells of immune mice and non-immune mice was carried out essentially as described recently (17).

*Northern hybridization.* Glyoxalated total RNA (20 $\mu$g per lane) was subjected to agarose gel electrophoresis (14) and transferred to a Hybond N membrane by a downward alkaline capillary transfer

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   1  AAG GAA CCC TGG TGT CTC AAT TCA GCA TCA GGT CGG CCT GTG TTC AGG ACC AGA GAC CCT
  61  GGT GAA CCC AGG AGT TAT AAA GGA ATA TTT TGT GAA ACG TCA GCA GGA GCA TCT GCA GGT
 121  GCA CAG AGA ATC CAA ATG CAG AAA GGA GAG ACG GGG AAG AAC CTG AGC TCA GAG AAC CCA
   1                       M   Q   K   G   E   T   G   K   N   L   S   S   E   N   P
                                                                   *
 181  AAG CAG ATG GGA GCA CCA GGC TTC CAA GGA GAA CAG GCA ATG TGG GTC CTT CCC TTG TAT
  16   K   Q   M   G   A   P   G   F   Q   G   E   Q   A   M   W   V   L   P   L   Y

 241  GCT GAA GGC TTG AAT ACA AGT CTT TCC CAG AGG AAA GCC TGT GTC TCT GAC TCA ATG CTG
  36   A   E   G   L   N   T   S   L   S   Q   R   K   A   C   V   S   D   S   M   L
                       *
 301  CCA CAT CTG ATC CTC AGG CTC AGA GGA CTC CAG GGC CCC GCA GAT GCC CCA GCT GAG GCT
  56   P   H   L   I   L   R   L   R   G   L   Q   G   P   A   D   A   P   A   E   A

 361  CAT CCT AGT GGG CAG GAC TGG GAC CGG CAA GAG TGC CAC TGG CAA CAG CAT CCT GGG TCA
  76   H   P   S   G   Q   D   W   D   R   Q   E   C   H   W   Q   Q   H   P   G   S

 421  GAA GTG CTT CCT GTC CAG GCT GGG GGC GGT GCC TGT CAC CAG AAG TTG CAC TTT GGC CAG
  96   E   V   L   P   V   Q   A   G   G   G   A   C   H   Q   K   L   H   F   G   Q

 481  CAG AAT GTG GGC CGG CTG GCA GGT GGA GGT GGT GGA CAC CCC GGA TAT CTT CAG CTC CGA
 116   Q   N   V   G   R   L   A   G   G   G   G   G   H   P   G   Y   L   Q   L   R

 541  GAT CCC GCG GAC CGA CCC TGG GTG CGT GGA GAC AGC CCG CTG CTT TGT GCT GTC GGC CCC
 136   D   P   A   D   R   P   W   V   R   G   D   S   P   L   L   C   A   V   G   P

 601  TGG GCG CAC GCG CTG CTG CTG GTC ACC CAG CTG GGT CGC TTC ACC ATG CAG GAC AGC CAG
 156   W   A   H   A   L   L   L   V   T   Q   L   G   R   F   T   M   Q   D   S   Q

 661  GCG CTG GCC GCG GTG AAG CGG TTA TTC GGG AAG CAG GTG ATG GCG CGC ACT GTC GTG GTG
 176   A   L   A   A   V   K   R   L   F   G   K   Q   V   M   A   R   T   V   V   V

 721  TTC ACG CGC CAA GAG GAC CTG GCT GGA GAC TCC CTG CAG GAT TAT GTG CAC TGC ACG GAC
 196   F   T   R   Q   E   D   L   A   G   D   S   L   Q   D   Y   V   H   C   T   D

 781  AAC CGC GCG CTG CGG GAC CTG GTG GCC GAG TGC GGG GGC CGC GTG TGC GCC CTC AAC AAC
 216 - N   R   A   L   R   D   L   V   A   E   C   G   G   R   V   C   A   L   N   N

 841  CGC GCC ACA GGC AGC GAG CGC GAG GCT CAG GCT GAG CAG CTG CTG GGC ATG GTT GCG TGC
 236   R   A   T   G   S   E   R   E   A   Q   A   E   Q   L   L   G   M   V   A   C

 901  TTG GTG AGG GAG CAC GGG GGC GCG CAC TAC TCC AAT GAG GTG TAT GAG CTG GTG CAG GAC
 256   L   V   R   E   H   G   G   A   H   Y   S   N   E   V   Y   E   L   V   Q   D

 961  ACG CGG TGC GCT GAC CCC CAG GAC CAA GTA GCC AAG GTG GCA GAG ATA GTG GCT GAG CGC
 276   T   R   C   A   D   P   Q   D   Q   V   A   K   V   A   E   I   V   A   E   R

1021  ATG CAG AGG CGC ACC AGG TTG CTA GCT GGG CTG TGG GGA TGG CGG AAA TTC TAC TGG AAG
 296   M   Q   R   R   T   R   L   L   A   G   L   W   G   W   R   K   F   Y   W   K

1081  GGC TGG AGG CGT GGT TTC TCT GTC TTC CTG GGT GTG GCC ATC TTG ATC TAC CTG CTG TTC
 316   G   W   R   R   G   F   S   V   F   L   G   V   A   I   L   I   Y   L   L   F

1141  TAC AGA AAG GGC TTT GGG GAC CAG AAT AAC AGA TAA AGC TGG TGT TAG AAG ATG TTC CGT
 336   Y   R   K   G   F   G   D   Q   N   N   R   -

1201  TTT TAA AAA AGA AAT AGT TAG AAT TCT GCT CAG AGC TTT AAT GAT TCA CAG TGC AGT GAC
1261  TTG GCA TCA ACT TTG CTA AGG TTC TGT TGT GTG AGG AGC GGG TGT GGC GGC AGT ACC AAA
1321  GGC ACC AGG GAC TGC AGC TAA GTC TTA TGG CTT GCA CCT GAC TTC CTC ATA CAC CTG AAT
1381  ATA AGC CAC AAA CAT CAT GAG AGC TGC ACA GGT GCA CCA GGA TAC TGG TGA ATC CAT TTT
1441  GAT GGA GAT ATG CCC CTG CTG CCC TGA TTA GCT GAA GCT GCG TGC CTG GTG AGG TGG CGT
1501  GGC CTG CTG TGG GTG GAT GGG AAC TGA GAG TAT ATA AGA GTG AGA GGC CCA GGG TTC GGG
1561  GGA GAT ATA AAC AAG AAG AAA GAT GAA GAC TGA ATA AAC TGC TGT TAG AAG G(A)n
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**FIG. 1.** Nucleotide and deduced amino acid sequence of *iap38*. The underlined amino acids represent putative transmembrane domains. Putative glycosylation sites are marked by asterisks. The polyadenylation signal is presented in bold letters.

procedure (18). Radiolabeled probes were generated by random labeling of linearized plasmid DNA using a Megaprime kit (Amersham, Braunschweig, Germany) and 50 $\mu$Ci [$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol). Hybridizations were done overnight at 65°C in 6 × SSC (1 × SSC: 0.15 M NaCl, 0.015 M Na-citrate), 5 × Denhardt's reagent (0.1% polyvinylpryrrolidone, 0.1% bovine serum albumin, 0.1% Ficoll 400), 0.1% SDS, and 100 $\mu$g/ml herring sperm DNA. Filters were washed under high stringency in 0.1 × SSC and 0.1% SDS at 65°C. A $\beta$-actin cDNA-probe was used as a control for RNA loading. Densitometric scanning of autoradiograms was performed using Imagemaster 1D software (Pharmacia, Freiburg, Germany). Integrated optical density over peak areas was normalized to corresponding actin signals and to that value obtained from the spleen of non-immune, non-infected mice not treated with testosterone.

*DNA sequencing.* Clones were sequenced using standard or sequence specific primers (Birsner und Grob, Denzlingen, Germany) by radioactive chain termination sequencing with the T7-sequencing kit (Pharmacia, Freiburg, Germany). Sequences were determined from both strands. Sequence data were analyzed with PC/GENE software (Intelligenetics, Mountain View, USA). DNA and protein databases of EMBL were used for on-line sequence comparisons (19).

*RACE-PCR.* Using the 5′ RACE kit from GIBCO BRL (Eggenstein, Germany) cDNA was synthesized from 1 $\mu$g spleen poly A$^+$ RNA with the primer 5′-ACC TGC TTC CCG AAT AAC CG and Superscript reverse transcriptase. The cDNA was C-tailed using terminal desoxynucleotidyl transferase. The first amplification was performed using 400 nM anchor primer and 400 nM of the primer 5′-TCC TGC ATG GTG AAG CGA CC in 50 $\mu$l containing 200 mM of each dNTP, 1.5 mM $MgCl_2$, one fifth of the tailed cDNA and 2.5 U Taq-polymerase. Initial denaturation was at 94°C for 5 min, followed by 35 cycles of 1 min 94°C, 1 min 50°C and 2 min 72°C, and final extension at 72°C for 10 min. The reaction conditions for nested PCR were the same with the exception that we used 1$\mu$l of the first amplification product as template and the gene specific primer 5′-AGC ACT TCT GAC CCA GGA TGC and 25 cycles with an annealing temperature of 55°C. The product was analysed on 2% agarose gels, eluted, and cloned into the TA-vector pMos (Amersham, Braunschweig, Germany).

## RESULTS AND DISCUSSION

Testosterone-resistant acquired immunity to *Plasmodium chabaudi* malaria can be mediated by spleen cells as we have previously revealed (7). Indeed, when spleen cells of immune C57BL/10 mice are transferred to non-immune, testosterone-immunosuppressed mice, the latter regain their capability of self-healing *P. chabaudi* malaria. In order to detect possible changes in gene expression of such immunity-mediating spleen cells, we used differential screening of a cDNA-library and isolated a 1.3 kb cDNA clone, which hybridizes with a 1.6 kb mRNA in northern blots. The 5′-region of this mRNA was isolated by 5′-RACE-PCR.

Fig. 1 shows the sequence of the full length cDNA clone. It is 1,612 bp long and contains an open reading frame with the start codon at position 136 and the stop codon at position 1,174. The polyadenylation signal begins at position 1,593. The sequence of the coding region does not reveal any significant homology to any other known sequence to date. The deduced amino acid sequence comprises a 38 kDa protein with a pI of 8.7 and is designated immunity-associated protein

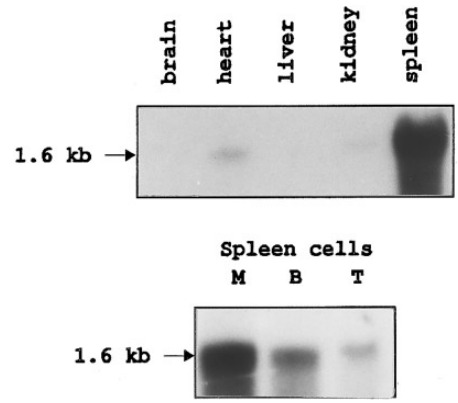


**FIG. 2.** Northern hybridization of *iap38*. Total RNA was isolated from tissues and spleen cells (M: macrophages; B: B cells; T: T cells) of immune C57BL/10 mice reinfected with *P. chabaudi* for 7 days. A cDNA fragment of *iap38* (position 319 to 1,226) was used as a probe.

(IAP38). Using PSORT (20), IAP38 is predicted to be a plasma membrane protein with two putative membrane-spanning domains from position 149 to position 167 and position 320 to 335, respectively (Fig. 1). Also, two N-glycosylation-sites are present at position 9 and 40, respectively.

Northern hybridization reveals that the mRNA of the *iap38*-gene is mainly expressed in the spleen of immune mice whereas only some minor expression occurs in other tissues of immune mice such as kidney and heart (Fig. 2). Expression of this gene is not detectable in liver and brain under the same hybridization conditions. In the spleen, *iap38* is expressed in both macrophages and B cells, and, to a lesser extent, in T cells (Fig. 2). Remarkably, the expression of *iap38* in the spleen strongly depends on the infectious and immune status of mice (Fig. 3). There is a low level expression in non-infected, non-immune mice. Infection for 7 days causes a slightly increased *iap38*-expression. This infection-inducible expression of *iap38* is obviously testosterone-sensitive, since a much higher expression is detected in spleens of testosterone-treated non-immune mice (Fig. 3). However, the most abundant expression of *iap38* occurs in spleens of immune mice. Thus, immune not re-infected mice express *iap38* at about 40-fold levels in comparison with non-infected, non-immune mice. This high constitutive expression levels of *iap38* appear to be not affectable by testosterone or re-infection with *P. chabaudi* malaria (Fig. 3).

Collectively, our data show that the acquisition of a testosterone-resistant, immunity-mediating phenotype of spleen cells is associated with a strongly enhanced constitutive expression of the novel gene *iap38*. This conspicious assosiation suggests that *iap38* is involved in those mechanisms which impart spleen cells the ability to resist the immunosuppressive activity of tes-

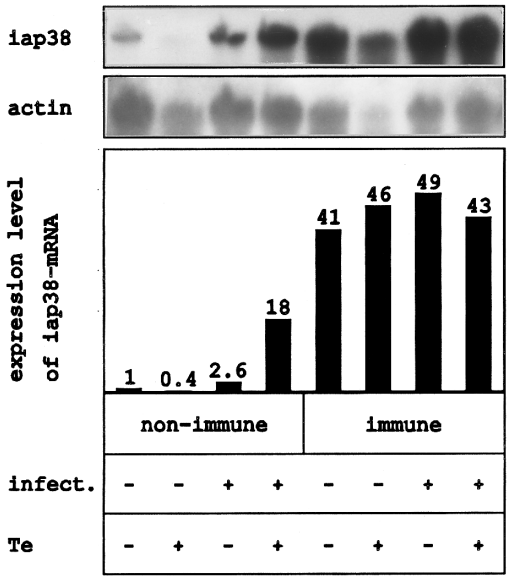


**FIG. 3.** Expression of *iap38* in the spleen of C57BL/10 mice. RNA was isolated from spleens of non-immune and immune mice with or without *P. chabaudi* infection (± infect.) and testosterone treatment (± Te) and analyzed by Northern hybridization. Quantitative evaluation of autoradiograms was done as described in Materials and Methods.

tosterone and to mediate protective immunity to *P. chabaudi* malaria.

## REFERENCES


1. Alexander, J., and Stimson, W. H. (1989) *Parasitol. Today* **4,** 189–193.
2. Grossmann, C. J. (1989) *J. Steroid Biochem.* **34,** 241–251.
3. Schuurs, A. H. W. M., and Verheul, H. A. M. (1990) *J. Steroid Biochem.* **35,** 157–172.
4. Helwig, M., and Wunderlich, F. (1987) *Europ. J. Cell. Biol.* **43,** 499–500.
5. Wunderlich, F., Mossmann, H., Helwig, M., and Schillinger, G. (1988) *Infect. Immun.* **56,** 2400–2406.
6. Wunderlich, F., Marinovski, P., Benten, W. P. M., Schmitt-Wrede, H.-P., and Mossmann, H. (1991) *Parasite Immunol.* **13,** 357–367.
7. Wunderlich, F., Benten, W. P. M., Bettenhäuser, U., Schmitt-Wrede, H.-P., and Mossmann, H. (1992) *Parasite Immunol.* **14,** 307–320.
8. Weiss, L. (1990) *Immunol. Letters* **25,** 165–172.
9. Benten, W. P. M., Bettenhäuser, U., Wunderlich, F., Van Vliet, E., and Mossmann, H. (1991) *Infect. Immun.* **59,** 4486–4490.
10. Wunderlich, F., Stübig, H., and Königk, E. (1982) *J. Protozool.* **29,** 60–66.
11. Wunderlich, F., Helwig, M., and Brenner, H. (1988) *Infect. Immun.* **56,** 3326–3328.
12. Benten, W. P. M., Wunderlich, F., Herrmann, R., and Kühn-Velten, N. (1993) *J. Endocrinol.* **139,** 487–494.
13. Miltenyi, S., Mueller, W., Weichel, W., and Radbruch, A. (1990) *Cytometry* **11,** 231–238.
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
15. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
16. Aviv, H., and Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
17. Willuhn, J., Schmitt-Wrede, H.-P., Greven, H., and Wunderlich, F. (1994) *J. Biol. Chem.* **269,** 24688–24691.
18. Chomczynski, P. (1992) *Anal. Biochem.* **201,** 134–139.
19. Lipman, D. J., and Pearson, W. R. (1985) *Science* **227,** 1435–1441.
20. Nakai, K., and Konekiro, M. (1992) *Genomics* **14,** 897–911.