

## SPECIFIC ANTI-*TOXOPLASMA GONDII* ANTIBODIES PRODUCED IN INBRED MICE DIFFERRING IN THEIR NATURAL RESISTANCE TO TOXOPLASMOSIS<sup>1</sup>

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**ABSTRACT.** The laboratory diagnostics of *T. gondii* infections both in humans and animals relies mainly upon detection of specific antibodies. We studied the influence of the host genetic factors on the level and repertoire of antibodies produced in *Toxoplasma* infection employing as an experimental model two inbred mouse strains with different innate susceptibility to toxoplasmosis. Using polyvalent antigen preparations in ELISA and microagglutination we found no differences in the antibody levels in both strains. By comparison of the antibody profiles in immunoblot we determined mouse strain-specific and common toxoplasmosis markers.

**Key words:** haplotypes, inbred mice, specific antibodies, toxoplasmosis.

### INTRODUCTION

The course of *Toxoplasma gondii* infection is dependent on many factors: virulence of parasite strain, route of infection, genetic background, age and immunological status of the infected hosts. In mice, in which the genetic dissection of host resistance is best described, many loci within MHC (H-2) and outside MHC controlling outcome of infection were identified (Johnson et al. 2002).

Because of intracellular replication of the parasite, the protective response is dominated by cell-mediated immunity. Generated antibodies are of limited value for host protection, however, they are very helpful in recognizing *T. gondii* infections by a variety of serological tests, which due to their relative availability and simplicity, are still the basis of toxoplasmosis laboratory diagnostics (Joynson and Guy 2001).

The aim of this study was to determine on experimental mouse model the influence of host genetic background (H-2 haplotype) on the level and specificity of antibody immune response and to search for typical antibody markers of acute and chronic toxoplasmosis.

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## MATERIAL AND METHODS

**Parasites and antigens.** Mouse–low virulent strain DX of *T. gondii* (intraspecies group II), maintained *in vivo* in inbred mice by intraperitoneal passages of cerebral cysts containing bradyzoites, was used to induce experimental infection. Strain BK (intraspecies group I) was utilized only for obtaining antigen preparations. BK tachyzoites were propagated *in vitro* in L929 mouse fibroblasts (variant aBK, attenuated) or *in vivo* by 2-fold passage in C57Bl/6 and BALB/c mice (variant vBK, virulent) (Długońska et al. 2001). An aqueous extract (TLA, *Toxoplasma* Lysate Antigens) was prepared from freeze-thawed parasites. From freshly collected tachyzoites ESA (Excreted/Secreted Antigens) preparation was obtained by 18h incubation of  $6 \times 10^8$  parasites/ml PBS supplemented with 2mM L-glutamine and 100 µg/ml gentamicin and protease inhibitor cocktail (Böhringer).

As controls in immunoblot assay four polyvalent antigen preparations were used: tachyzoites of tightly related apicomplexan parasite *Neospora caninum*, non-related bacterial antigens (glycine extract of *Helicobacter pylori*, sonicate of *Listeria monocytogenes*) and lysate of L929 mouse fibroblasts as host cells for *T. gondii* BK growing *in vitro*.

**Mice.** Inbred mice, 3-5 month-old males, differing in their innate resistance to toxoplasmosis: C57Bl/6 (H-2<sup>b</sup> haplotype, relative susceptible) and BALB/c (H-2<sup>d</sup> haplotype, relatively resistant) were used. Mice were infected intraperitoneally with 5 cerebral cyst of *T. gondii* DX and at different time post infection (1 week, 3 weeks and 2 months) blood probes were taken. Each experimental mouse group consisted of 6 individuals. The study was approved by the Local Ethical Council in Łódź (Ł/BD/72, 11.10. 2001).

**ELISA** for estimation of anti-*T. gondii* antibodies was performed as described earlier (Długońska et al. 2001) with mouse sera diluted: 1:100 (normal and 1-week post infection sera), 1:500 (3-week sera) and 1:3000 (2-month sera), as estimated in preliminary experiments. The results were expressed as mean of arbitrary units (calculated from absorbance values x dilution)  $\pm$  SD; cutoff values (mean values + 3 SD) were calculated for individual antigen preparations using mouse sera of non-infected mice.

**Microagglutination assay.** Acetone (A) and formaline (F)-fixed parasite (*T. gondii* BK) cell suspensions, prepared according to Dannemann et al. (1990), were used to determine antibody titers in sera two-fold diluted, from 1:125 to 1:128 000.

**Immunoblot** to compare antibody profiles was performed in reducing (2-ME+) conditions with vBK antigen preparation (TLA vBK) as described previously (Długońska et al. 2001).

**Statistics.** The results of ELISA were expressed as arithmetic means ( $n=6$ )  $\pm$  SD and of microagglutination assay as log<sub>10</sub> titer reciprocals ( $n=6$ ). Statistical significance was evaluated using Mann-Whitney U-test ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

The level and repertoire of specific antibodies in mice infected with *T. gondii* DX were determined during acute toxoplasmosis (1 week of postinfection), on the turn of the end of acute and the beginning of chronic toxoplasmosis (3 weeks) and in chronic toxoplasmosis (2 months) with heterologic antigen preparations of BK strain origin. The time points of testing were chosen on the basis of preliminary experiments, showing that cerebral cysts as markers of chronic toxoplasmosis appeared after 2 weeks of infection and immunosuppression persisted in innately susceptible mouse strains to the end of 3<sup>th</sup> week.

The infected mice were shown to be positive in ELISA after 1 week (Fig.1 a). The antibody level increased significantly at week 3 and much more intensively at 2 months of postinfection. There were no differences in immune response intensity of susceptible and resistant to toxoplasmosis mice ( $p > 0.05$ ).

The activity of all sera tested in ELISA was evaluated also in microagglutination assay performed with both acetone- and formaline-fixed toxoplasma cells, the use of which can discriminate between „early” IgG antibodies reacting with acetone-treated toxoplasms and „late” IgG antibodies reacting with formaline-treated parasites. There were not any statistically significant differences ( $p > 0.05$ ) in the levels of specific agglutinating antibodies generated in both mice strains (Fig.1b).

The immunoblot allowed comparative analysis of developed antibodies. The first ones, which appeared at week 1 post infection in both mouse strains, were directed against 38 kDa antigen fraction. They were accompanied by anti-30 kDa and followed by anti- 24, 33 and 97 kDa antibodies. These 5 specificities are common markers of acute toxoplasmosis (Fig. 2, <). In chronic toxoplasmosis antibodies against 22, 46, 48, 58, 86, 97 and 104 kDa antigen fractions joined. Additionally, some mouse strain – specific markers could be appointed: for BALB/c – 16, 18, 27, 70 kDa and for C57Bl/6 – a cluster 40, 41, 42, 43 kDa (Fig. 2, <<). It is worth mentioning that the sera of non-infected (control) mice were absolutely negative and no bands typical for *Toxoplasma* infection were found in any other controls with non-*T. gondii* antigen preparations (not shown).

On a model of experimental toxoplasmosis in inbred mouse strains differing in their natural susceptibility to *T. gondii* infections we determined that genetic background (MHC, H-2 haplotype) influenced, to some degree, only the profile but not the total level of produced antibodies. Previous studies carried out on mice (Appleford and Smith 2000) or humans (Huskinson et al. 1989, Meek et al. 2001) demonstrated *T. gondii* antigens predominantly recognized by antibodies but did not analyze antibody specificity in respect to genetic traits of infected host.

Summarizing our results from mouse and human (Długońska et al. 2001, Dytnerka et al. 2003a) systems – despite the varied profile of antibody immune response, which was species, strain and individual – dependent, 4 common sero-

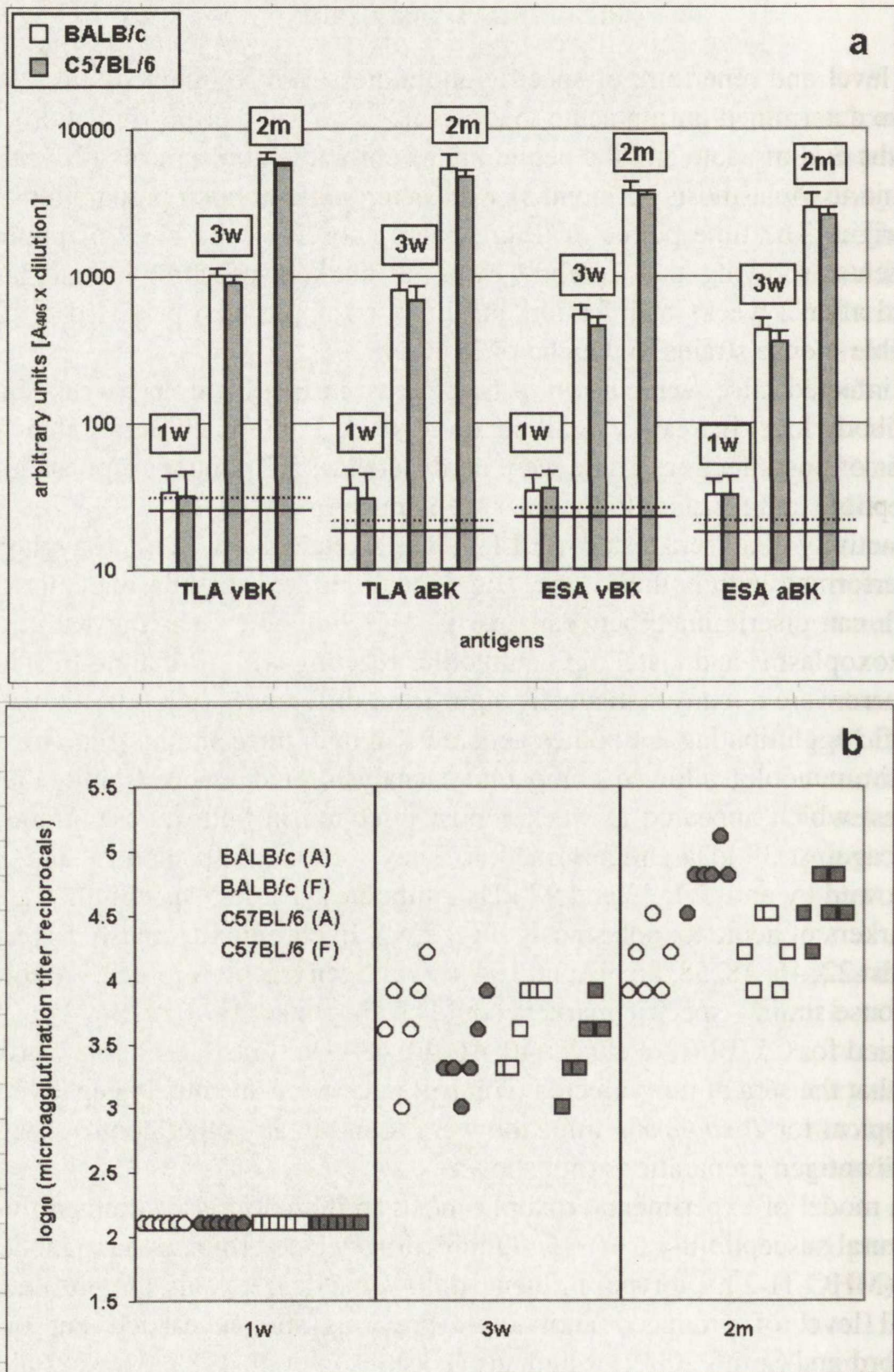


Fig.1. The levels of specific antibodies in mice infected with *T. gondii* DX: 1 week (1w), 3 weeks (3w) and 2 months (2m) post infection; a – ELISA: cutoffs for BALB/c (....), C57Bl/6 (-), b – microagglutination: (A) – acetone-fixed, (F) – formaline-fixed, geometric means (-), ↓ – the titer < 2,097 (<1:125)

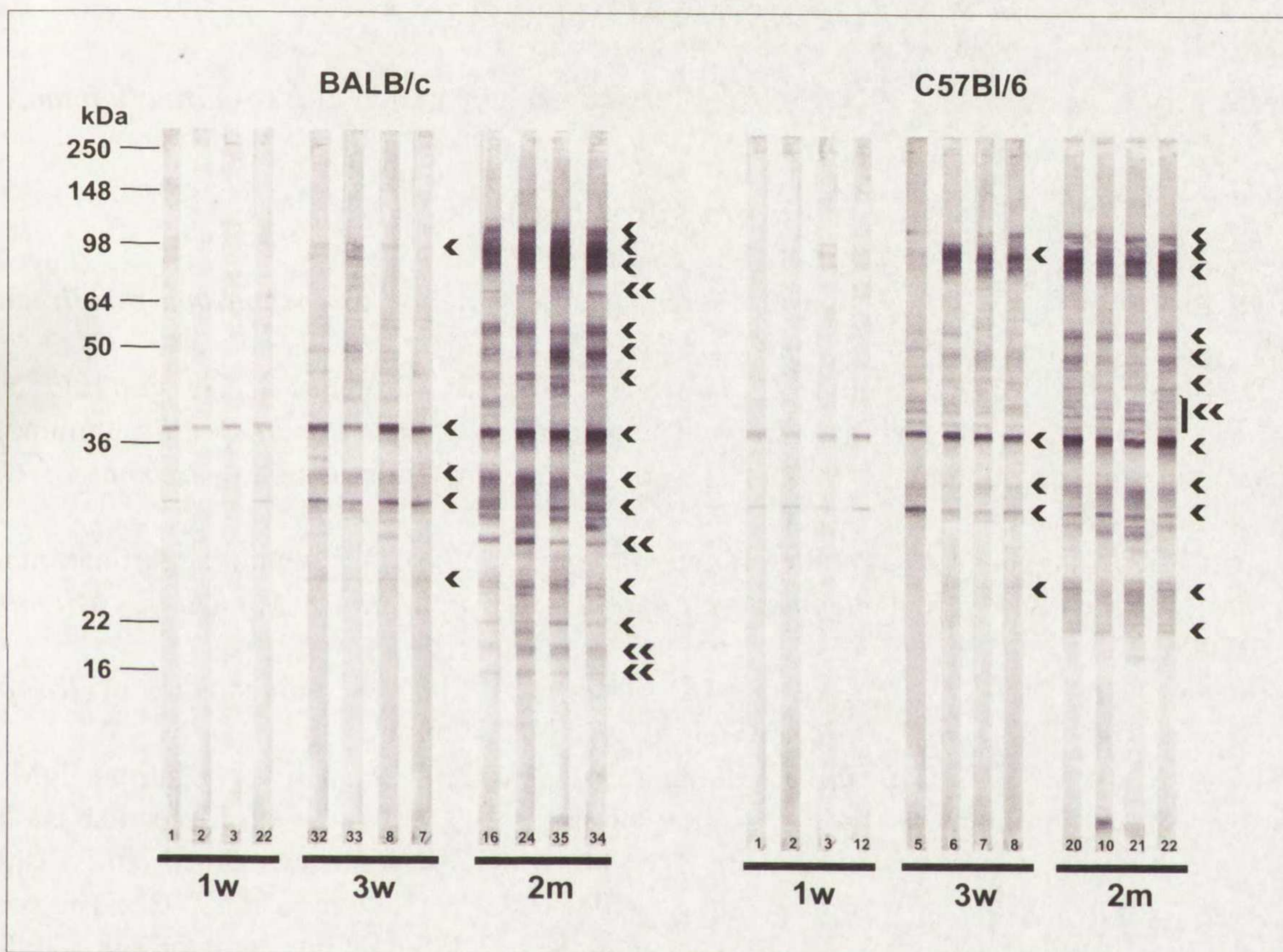


Fig.2. Specific antibody profiles in BALB/c and C57BL/6 mice infected with *T. gondii* DX; 1 week (1w), 3 weeks (3w) and 2 months (2m) post infection; common (<) and mouse strain-specific (<<) immunodominant antigens; a cluster of 41, 42, 43, 44 kDa antigens (I <<)

logical markers of toxoplasmosis were identified: immunodominant antigen fractions of 30, 33, 38 and 58 kDa. Besides, in recently described case of congenital toxoplasmosis two early markers of acute infection – 30 and 38 kDa – were detected (Dytnerska et al. 2003 b). The nature of both is not known yet. The signal at 30 kDa observed on immunoblots with reduced antigen preparation (Fig. 2) is not attributed to anti-SAG1 antibodies, because they stain SAG1 (major surface tachyzoite antigen) in non-reducing conditions only (data not shown).

Because incompletely defined *Toxoplasma* lysate as antigen source may cause reproducibility problems, it is important to identify in further investigations individual antigens located in immunodominant fractions and then use in immunoblot a standard set of recombinantly produced antigens instead of single antigens, which alone can be not suitable for diagnostic assays (Meek et al. 2001). Aubert et al. (2000) by evaluation the diagnostic utility of 11 recombinant antigens (inclusive SAG1) found that no single recombinant antigen ELISA was able to detect all human IgM- or IgG- positive probes, therefore the determination of diagnostic antigens set seems to be of great importance for specific serological recognition of toxoplasmosis.

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