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Orientation of bovine CTL responses towards PIM, an antibody-inducing surface molecule of *Theileria parva*, by DNA subunit immunization

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Abstract

East Coast fever, an acute lymphoproliferative disease of cattle, is caused by the apicomplexan parasite *Theileria parva*. Protective immunity is mediated by $CD8^+$ cytotoxic T lymphocytes directed against schizont-infected cells. The polymorphic immunodominant molecule, although an antibody-inducing surface molecule of the schizont, has been hypothesized to play a role in protective immunity. In order to evaluate the immunogenicity of PIM for inducing CTL, cattle were immunized with PIM in isolation from other *T. parva* antigens, forcing the presentation of PIM-derived epitopes on the MHC class I molecules. Although parasite-specific cytotoxicity was induced in both vaccinated animals, their immune response was clearly different. One animal generated MHC-restricted parasite-specific CTL against PIM while the other calf exhibited a strong PIM-specific proliferative response but non-MHC-restricted parasite-specific cytotoxicity. Only calf 1 survived a lethal sporozoite challenge. This DNA immunization technique with an antigen in isolation of CTL-immunodominant antigens might open possibilities for directing CTL responses against predefined antigens, such as strain cross-reacting CTL antigens.

Keywords: $CD8^+$ T lymphocytes; *Theileria parva*; DNA vaccine; Cattle

1. Introduction

Theileria parva is a tick-borne apicomplexan parasite causing East Coast fever (ECF), a fatal lymphoproliferative disease of cattle. Upon tick feeding, sporozoites rapidly invade lymphocytes were they develop into multinuclear schizonts and cause the infected host cells to transform into immortalized lymphoblasts (Hulliger et al., 1964). Susceptible cattle almost invariably die within 2–4 weeks post-infection due to a metastatic invasion of vital organs and/or the widespread destruction of lymphoid tissues (Emery, 1981). Cattle can be vaccinated by infection with live *T. parva* sporozoites and treatment with theilericidal drugs (Neitz, 1953). This infection and treatment regimen provides protection against the immunizing parasite strain but not always against heterologous strains (Irvin, 1987; Young et al., 1973).

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By in vitro re-stimulation of immune PBMC with autologous infected cells, it is possible to generate CTL lines and clones that are parasite strain-specific and MHC class I-(MHC-I) restricted (Goddeeris et al., 1986b,c). Specific CTL responses were also shown in vivo in animals undergoing immunization and their appearance coincided with clearance of the infection (Morrison et al., 1987). Moreover, the parasite strain-specificity of the CTL correlated with cross-protection in cattle (Taracha et al., 1995a) and their protective effect could be adoptively transferred with the $CD8^+$ population (McKeever et al., 1994). Quite importantly, strain cross-specificity of CTL (and consequently cross-protection) was not only determined by the immunizing strain, but also by the restricting MHC-I haplotype, indicating that the latter presents different epitopes depending on the immunizing strain (Goddeeris et al., 1990; Taracha et al., 1995b). From all this data it is clear that CTL play an important role in the protection against ECF and that subunit vaccines should target proteins containing CTL epitopes.

Recently, Graham et al. (2006) identified six candidate CTL antigens by both targeted and random immunoscreens of schizont cDNA-transfected skin fibroblasts with CTL from immune animals. Vaccination of naive cattle with 5 of these antigens, either as DNA prime/virus boost or a heterologous virus prime/boost, readily induced an antigen-specific $CD8^+$ T cell IFN-γ response, but CTL could only be detected in 17% of the vaccinated animals and an additional 12.5% after challenge. Interestingly, the presence of a parasite-specific CTL response correlated with protection against a lethal challenge. Marginal T helper responses were detected in 39% of the vaccinated animals and these did not correlate with the presence of CTL.

Conversely, the polymorphic immunodominant molecule (PIM), a schizont surface protein and not present among the 6 CTL candidate antigens, is one of the most immunogenic antigens of *T. parva*, at least for the induction of antibody responses (Toye et al., 1991, 1995b, 1996). The antigen is highly expressed, both by schizonts and sporozoites (Bishop et al., 2005), and has been hypothesized to play a role in protective immune responses against *T. parva*, mostly based on its genetic diversity and the unusual organization of the PIM gene (Geysen et al., 2004). The gene consists of a variable central region flanked by two conserved termini, with two conserved introns in the 3′ conserved region (Toye et al., 1995a). Recently, it was shown that the polymorphism of the coding sequence shows a mosaic pattern (Geysen et al., 2004). Since PIM is encoded by only one gene per haploid genome, the polymorphism is due to a rapid evolution of the coding sequence, possibly under pressure from the host's immune system.

The objective of this study was to test whether PIM can induce CTL responses in cattle. Therefore, a vaccination approach using plasmids expressing PIM in isolation of other *Theileria* antigens was chosen, thus avoiding competition with CTL-immunodominant epitopes from other *Theileria* antigens. This approach contrasts with the strategy to identify CTL antigens in animals immunized by infection and treatment (Graham et al., 2006), where only the most immunodominant epitopes for a given MHC are targeted.

2. Materials and methods

2.1. Animals and MHC-I-typing

Three Belgian red-white milk type calves, 10–14 months old, were used for this study. The calves were MHC-typed by amplification and sequencing of the polymorphic exon 2–exon 3 region of MHC-I cDNA. Total RNA was extracted from PBMC (see below) with TRIzol according to the manufacturer's instructions. First strand cDNA synthesis was done with Omniscript (Qiagen, Germany) with a reverse primer binding at the end of exon 3 (5′-TCC AGG TAT CTG CGG AGC-3′), after which the polymorphic region was amplified with the same reverse primer and a forward primer at the start of exon 2 (5′-GCT CCC ACT CBM TGA GGT ATT-3′; with B = C/G/T and M = A/C) (Sawhney et al., 2001). Amplification of the cDNA was carried out by PCR with Pfu polymerase (Stratagene, USA). Amplification was accomplished by denaturation at 95 °C for 1 min, annealing at 63 °C for 30 s, and extension at 72 °C for 1 min and 30 s for 35 cycles.

The amplified fragments were purified from a 2% agarose gel and cloned in the pPCRScript Amp SK+ cloning vector (Stratagene), after which clones containing a single expressed class I MHC region were sequenced with the ABI PRISM Bigdye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA), using the above primers and the vector-specific primer T3 (5′-AAT TAA CCC TCA CTA AAG GG-3′). The sequences were aligned using DNAman software (Lynnon, Canada) and compared to the known cattle MHC-I alleles in the IPD-MHC sequence database (Robinson et al., 2005). One new allele was identified and submitted to the National Center for Biotechnology Information (GenBank), http://www.ncbi.nlm.nih.gov (accession number DQ786580).