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Cellular and humoral recognition of *Toxoplasma gondii* P30 antigen and its cleavage fragments

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tal loss due to this disease are of great economic importance worldwide. Infection with *T gondii* occurs most commonly *via* the oral route and induces specific IgA synthesis in the gut and milk secreting glands. The target antigens for these IgA have been already identified (Chardès *et al*, 1990) and the gene encoding for one of these antigens (dense granule protein Gra 4) has been sequenced (Mevelec *et al*, submitted). Most evidence indicates that cell-mediated immunity plays a central role in resistance to toxoplasmosis. Besides the local IgA response, further insight into the ability of *T gondii* antigens (P30, Gra 4 and the 142–156 peptide from Gra 4) to orally induce a potent local immune response may be gained by comparative studies of the intestinal and splenic T cell response. Following oral infection of C57B16 mice, an early T lymphocyte blastogenic response to a toxoplasma sonicate (TSo) occurred on day 6 with mesenteric lymph node (MLN) cells; this response then rapidly decreased. On the other hand, no or low blastogenic stimulation was shown with T lymphocytes from Peyer's patches (PP). A later splenic (SPL) blastogenic response began during week 4, peaked at week 6 and persisted throughout the experiment (13 weeks). MLN and SPL lymphocyte phenotypes showed a decrease in L3T4/Lyt2 ratio during murine toxoplasmosis. Subsequent experiments were performed at the peak of cell stimulation. Oral infection of Balb/c (H-2^d), CBA (H-2^k) and C57B16 (H-2^b) mice with *T gondii* cysts allowed us to define H-2^k as the higher responder haplotype for the MLN and SPL T cellular immune response to TSo. Cellular immune response to P30 and Gra 4 was restricted to the H-2^k and H-2^d haplotype, whereas 142–156 peptide only induced a T cell response under H-2^k haplotype restriction. Finally, using the CBA high responder model, lymphocyte proliferation was associated in most cases with γ IFN and IL6 production but not with IL2 secretion. No definitive conclusion can be made concerning a correlation between the cytokine pattern and a particular Th subset. Further analysis, such as evaluation of IL4 and IL5 production, might clarify this point.

In conclusion, in addition to the induction of a mucosal IgA response, P30 and Gra 4 are able to stimulate an early local cellular immunity and a later systemic cellular response under H-2 restriction. Using the oral infection model, the 142–156 peptide derived from the deduced amino-acid sequence of Gra 4 seems to bear an H-

2^k restricted T epitope. The cellular immune characterization of such antigens is a further step leading towards an orally-induced immune protection against toxoplasmosis.

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Cellular and humoral recognition of *Toxoplasma gondii* P30 antigen and its cleavage fragments. Velge-Roussel F, Chardès T, Mevelec P, Bout D (UFR des Sciences Pharmaceutiques de Tours et Laboratoire INRA de Pathologie Infectieuse et d'Immunologie, Unité de Recherche Université Tours-INRA d'Immunologie Parasitaire, 37380 Nouzilly, France)

Cell mediated immunity is a major mechanism of protection in toxoplasmosis. *T gondii* has been shown to stimulate T cell subsets *in vitro* with cytokine release and to activate NK cells following infection. The predominant parasite antigens able to induce this T cell stimulation remain largely unknown due in part to the lack of purified antigens. The P30 is one major *T gondii* protein which is well known as diagnostic antigen in human acquired toxoplasmosis. Moreover, P30 has been shown to induce *in vitro* polymorphonuclear blood lymphocyte (PBL) proliferation in infected patients. In mouse infection, beside the high level of specific Ab in sera, we have also found local specific antibody in milk and intestinal secretions (Chardès *et al*, 1990). Finally, specific cytotoxic T cells (Lyt2⁺) were demonstrated as able to kill tachyzoites *in vitro*. These data indicate that P30 antigen could be a suitable candidate for T and B epitope studies and

determination of the processing pathway. *T gondii* antigens capable of inducing a T cell immune response must be first processed to be CMI inducers. It is generally accepted that antigen processing often involves mild proteolysis and that the resulting protein fragments may bind to MHC products for subsequent presentation to T cells (Buus and Wederlin, 1986).

The purified P30 was cleaved on methionine residues using the cyanogen bromide method. Three major fragments were obtained with an apparent molecular mass of 19, 17, 14 kDa (F1, F2 and F3, respectively). The N terminal portion of the fragment F1 was sequenced and 6 amino acid were identified. Humoral recognition was supported by the fact that polyclonal anti-P30 Abs in mouse and infected human sera revealed the 19-kDa fragment. We were further interested in the cellular response to these fragments. Specific anti-P30 T cells were induced by immunisation of various strains mouse such as Balb/c (H2-d), CBA (H2-k) and C57B16 (H2-b) with purified P30. These experiments allowed us to define Balb/c (H-2d) as the higher responder haplotype. Fragment recognition was obtained for F1 with CBA cells and for F2 with Balb/c cells. The cytokine pattern was determined using bioassays for IL2 and IL6 and using an ELISA test for γ interferon. P30 seems able to induce low levels of IL2 and high levels of IL6 except with CBA for IL6 and B6 cells for IL2. The γ interferon secretion appears more variable. Experiments have been carried out with human PBL to determine immune fragment recognition. Preliminary results with only a few patients show that the P30 is recognized by 2 out of 3 infected humans. For patients who recognize P30, cell proliferation with F1 is obtained in one case, but more experiments are needed before a definitive conclusion can be made on the human recognition of these fragments.

In conclusion, the data show that the Balb/c is the high responder haplotype for P30 in our immunisation model. This immunogenicity is correlated with cytokine release: a low level of IL2 and a high level of IL6. The F1 and F2 fragments seem to share T epitopes in a mouse model with haplotype restrictions. We can suppose the existence of at least one B epitope on F1. It may also share one human T epitope. No definitive conclusions can be made concerning the cytokine pattern and a particular Th subset. IL4 and IL5 assesement might clarify this problem, and their evaluation is now under investi-

gation. This work is a preliminary step for the study of processing of the P30 toxoplasma antigen. Using the same protocol, we now envisage investigating natural processing pathways with purified cathepsins.

References

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Les sous-populations lymphocytaires alvéolaires et leur état d'activation au cours de la pneumonie interstitielle du mouton induite par le lentivirus Visna-Maedi. JI Cadore¹, F Guiguen¹, G Cozon², JF Mornex², G Cordier² (¹ INRA, laboratoire associé pathologie des petits ruminants, École vétérinaire, Marcy-l'Étoile; ² INSERM U80, laboratoire d'immunologie et de biologie pulmonaire et service de pneumologie, Lyon, France)

Les lésions de pneumonie interstitielle au cours de l'infection spontanée et expérimentale du mouton par le virus Visna-Maedi sont caractérisées par une alvéolite macrophagique endoluminale, une infiltration des septa interalvéolaires par des cellules mononucléées, une myomatose et la présence de modules lymphoïdes pérbroncho-vasculaires parfois intraparenchymateux. Les macrophages alvéolaires sont les cellules cibles du virus (Mornex *et al*, 1990). L'analyse en cytométrie en flux des macrophages alvéolaires recueillis par lavage bronchio-alvéolaire traités avec des anticorps monoclonaux anti-classe II spécifiques et la recherche de la libération par les macrophages de médiateurs témoins de leur état d'activation montrent qu'il existe une activation des macrophages alvéo-