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Research paper

The use of binding-prediction models to identify *M. bovis*-specific antigenic peptides for screening assays in bovine tuberculosis

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ABSTRACT

The identification of MHC class II-restricted antigenic peptides for inclusion into vaccines and/or as diagnostic test reagents for mycobacterial infections remains a high research priority. To expedite discovery of such peptides, numerous bioinformatic tools have been developed to predict whether a given peptide is likely to form a stable binding interaction with MHC class II molecules. However, no prediction tool dedicated to the identification of bovine MHC (BoLA) class II-restricted peptides is currently available. Using experimental immunogenicity data derived from the stimulation of whole blood of *Mycobacterium bovis*-infected cattle with 105 individual *M. bovis*-derived peptides, we have compared the ability of a novel BoLA DRB3 structure-based prediction method (Hepitom) with the human MHC class II binding predictor model ProPred in predicting peptides that induce bovine T-cell activation. When a stringent cut off for considering peptide antigenicity was applied, the sensitivities of Hepitom and ProPred in detecting immunogenic peptides were 62% and 77%, respectively. In contrast, the Hepitom model showed greater specificity, with values of 66% and 34% for Hepitom and ProPred, respectively. Using all peptides, seven out of eleven *M. bovis* proteins were identified as being highly immunogenic. All but one of these antigens were also identified when just the Hepitom predicted peptides were used, while only four of the seven were identified using the ProPred predicted peptides. In conclusion, we demonstrate that the Hepitom model is a useful pre-screening tool to select peptides for further immunogenicity studies in cattle without major impact on the identification of antigenic *M. bovis* proteins.

1. Introduction

Despite the current 'test and slaughter' control policy, the incidence of bovine tuberculosis (TB) caused by *Mycobacterium bovis* (*M. bovis*) has steadily increased in Great Britain over the last twenty years (DEFRA, 2010). Thus, the formulation of an effective vaccine to help control the spread of bovine TB infection remains a high research priority. Although *M. bovis* bacillus Calmette-Guerin (BCG), the only available vaccine to date for bovine TB, has shown varying efficacy in cattle (Berggren, 1981; Buddle et al., 1995; Waddington and Ellwood, 1972), encouraging results from recent studies have demonstrated that BCG vaccination efficacy can be significantly enhanced following boosting with viral, protein or DNA subunit vaccines (Skinner et al., 2005; Vordermeier et al., 2006, 2004, 2009; Wedlock et al., 2005). However, BCG vaccination results in sensitization to bovine tuberculin purified protein derivative (PPD-B) and compromises the current tuberculin-based skin test used for diagnosis of bovine TB.

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Therefore, the search for alternative immunogenic proteins and peptides for use as vaccine candidates and/or as reagents in diagnostic tests able to differentiate *M. bovis* infected and uninfected-vaccinated animals remains a key issue in bovine TB research.

It has long been known that CD4+ helper T-cells play an essential role in the control of mycobacterial infections through their production of the cytokine IFN-γ and its subsequent activation of macrophage microbicidal pathways (Boom, 1996; Orme and Cooper, 1999). The importance of this arm of the immune response has been demonstrated in small-animal studies, where mice deficient in IFN-γ fail to control infection with a sub-lethal challenge of M*ycobacterium tuberculosis* (*M. tuberculosis*) (Cooper et al., 1993). In order to become activated, CD4+ T-cells must first receive appropriate signals from specialised antigen presenting cells (APC) presenting peptides containing immunogenic epitopes in the context of major histocompatibility complex (MHC) class II molecules. The classical way of identifying these immunogenic epitopes is through the experimental screen of libraries of overlapping peptides spanning the protein of interest. However, this approach is often time-consuming and expensive, especially for large antigens. Given that the binding of antigenic peptides to the MHC class II molecule is considered to be a highly selective step in the antigen processing/presentation pathway, numerous bioinformatic tools have been developed to predict whether a given peptide is indeed likely to form a stable binding interaction with MHC class II (reviewed in Lafuente and Reche, 2009). Although the prediction accuracy for some of these computational methods has been reported to exceed 80% (Lin et al., 2008), other studies are more cautious (Gowthaman and Agrewala, 2008; Wang et al., 2008). What appears to be a consensus, however, is that the prediction accuracy is dependent on the experimental data sets used and that these current methods require a large amount of high-quality experimental binding data which are still far from sufficient, even in human studies.

Despite controlled breeding programs, cattle still exhibit high MHC diversity (Ellis and Ballingall, 1999). The highly polymorphic bovine MHC class II complex (BoLA) consists of one DR gene pair (comprised of monomorphic DRA and polymorphic DRB3) and one or two polymorphic DQ gene pairs expressed per haplotype (reviewed in Lewin et al., 1999). In contrast to other species (e.g. humans and mice), experimental peptide-binding data for BoLA class II-restricted peptides is severely lacking, and no computational prediction tools are currently available dedicated to the identification of BoLA class II-restricted peptides. This being said, we have previously demonstrated some success of the human prediction program ProPred (http://www.imtech.res.in/raghava/ProPred (Singh and Raghava, 2001)) in identifying peptides recognised by bovine T-cells (Vordermeier et al., 2003).

In the study presented herein, we describe a novel structure-based prediction method (Hepitom) based upon evaluating the binding free energies between BoLA DRB3 and a panel of peptides derived from the sequences of 13 *M. bovis* antigens. Using immunogenicity data derived from the stimulation of whole blood from *M. bovis*-infected cattle with 105 individual overlapping peptides, we have evaluated and compared the sensitivity and specificity of both the Hepitom and the ProPred models in predicting peptides that induce bovine T-cell activation.

2. Materials and methods

2.1. Cattle

All animals were housed at the Veterinary Laboratories Agency at the time of blood sampling and procedures were conducted within the limits of a United Kingdom Home Office Licence under the Animal (Scientific Procedures) Act 1986, which was approved by the local ethical review committee. Heparinized blood samples were obtained from 18 naturally infected, SICTT-positive reactors in herds known to have bovine TB as determined by the Animal Health Agency. Heparinized blood samples were also obtained from 4 animals who were experimentally infected ca. 6 months earlier with an *M. bovis* field strain from Great Britain (AF 2122/97) by intratracheal instillation of 1×10^3 CFU as previously described (Dean et al., 2005). A detailed post mortem examination of the TB-reactor animals revealed visible TB-lesions in all but two animals, confirming the presence of active disease.

2.2. Production and preparation of peptides

Overlapping peptides representing antigens Rv3875 (ESAT-6) and Rv3615c were prepared as previously described (Sidders et al., 2008; Vordermeier et al., 2003). Eleven secreted, or potentially secreted, proteins (Rv1037c, Rv1038c, Rv1197, Rv1198, Rv1792, Rv1793, Rv1860, Rv2346c, Rv3020c, Rv3803c and Rv3890c) were selected for antigen screening studies as previously described (Jones et al., 2010). Peptides representing these antigens were commercially synthesised as 20-mers overlapping by 12 amino acids (Mimotopes Pty Ltd., Clayton, Australia), dissolved in RPMI 1640 containing 20% DMSO to obtain a concentration of 5 mg/ml and used individually to stimulate whole blood at a final concentration of 10 μg/ml.

2.3. IFN-γ enzyme-linked immunosorbent assay (ELISA)

Whole blood aliquots (250 μl) were added in duplicate to antigen in 96-well plates and incubated at 37 °C in the presence of 5% CO_2 for 24 h, following which plasma supernatants were harvested and stored at −80 °C until required. Quantification of IFN-γ in the plasma supernatants was determined using the Bovigam ELISA kit (Prionics AG, Switzerland). A result was considered positive if the optical density at 450 nm (OD_{450}) with antigen minus the OD_{450} without antigen was ≥0.1 in both of the duplicate wells (Jones et al., 2010).