Summer Studentship Report

Student: Ben Somerton
Supervisors: Prof. Frank Griffin
Dr Sven Berger
Date: 13 March 2005

ISOLATING ANTIBODIES WHICH RECOGNISE PARATUBERCULOSIS ANTIGENS

Introduction

'Antibody phage display' is a powerful and innovative technique used to isolate antibodies which recognise target antigens. A sheep single chain antibody library was screened against a library of singly isolated *M. paratuberculosis* antigens.

The sheep antibody library was prepared as follows: Spleen cells were extracted from a sheep which was chronically infected with *M. paratuberculosis*. Gene segments within the spleen cells, which encode for the variable, binding region of antibodies, were amplified and inserted into phages. This was done in such a way that the phages displayed the single chain antibodies on their surface, so have the ability to bind to antigens recognised by the single chain antibody. Phages that displayed antibodies which recognised Mycobacteria antigens were amplified within the library. Phages that displayed antibodies which recognised irrelevant antigens, such as *E. coli* and phage proteins, were removed from the antibody library. Thus we were able to produce a mixture of antibodies which are highly specific to *M. paratuberculosis* antigens.

The *M. paratuberculosis* antigen library was prepared as follows: Copies of the *M. paratuberculosis* genome were 'cut up' into fragments containing around one gene per fragment. These genes were inserted into phages (different phages to those used to produce the antibody library). Phage clones were isolated and allowed to infect *E. coli* cultures. Each *E. coli* culture produced multiple copies of one *M. paratuberculosis* antigen.

A novel methodology was set up which allowed us to isolate antibodies which recognised *M. paratuberculosis* antigens while also being able to identify and characterise the antigen in the process.

Achievements

Hundreds of single chain antibody clones and around 1000 paratuberculosis antigens were isolated. 36 single chain antibodies were screened against 192 paratuberculosis antigens and were analysed in an ELISA. Two ELISA plates were used (Plates A and B), each containing 96 different paratuberculosis antigens. For the screening procedure the antigens from each of the plates were pooled, making two antigen mixtures called Plates A and B (Figure 1). An additional antigen mixture was used which contained *M. paratuberculosis* cell lysate, so that we could investigate whether the antibodies would have the ability to specifically recognise their target antigens

from a core mixture of *M. paratuberculosis* antigens. Several antibodies reacted strongly with the antigen they recognised. Four antibodies were selected for further analysis (Figure 1), each called Ab 11, 28, 30 and 34. Characteristics of an 'ideal' antibody would be one which reacts with the *M. paratuberculosis* cell lysate and one of either Plates A or B. The four antibodies investigated in Figure 1 displayed 'ideal' characteristics, especially Abs 28 and 34.

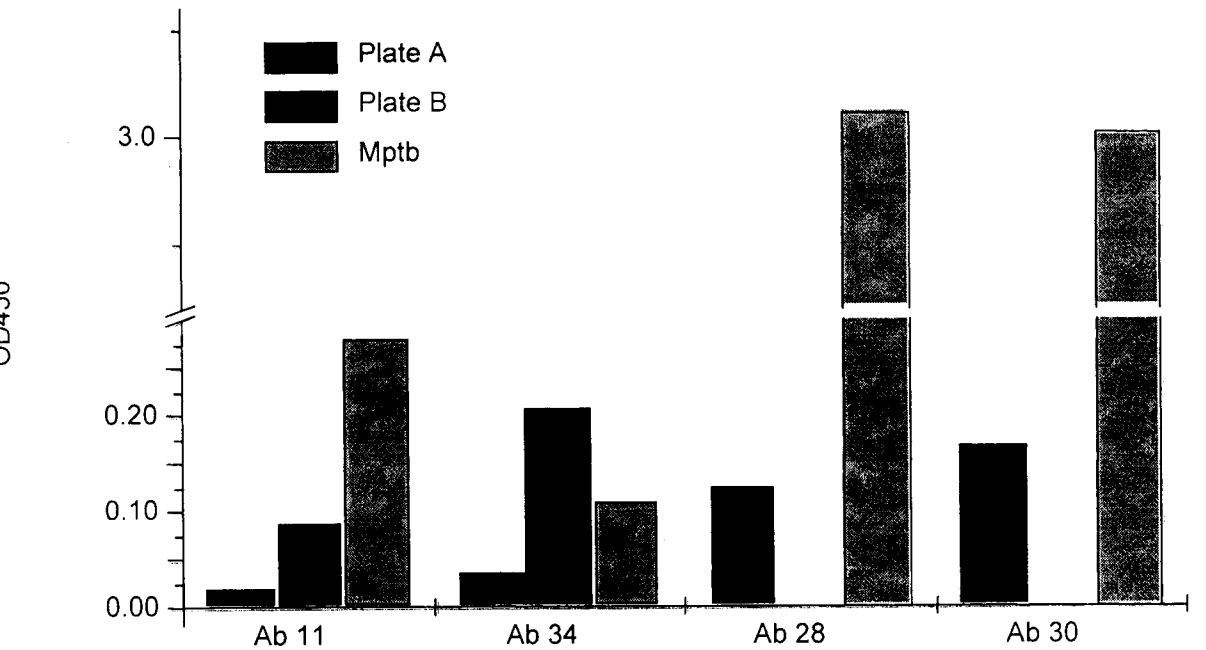
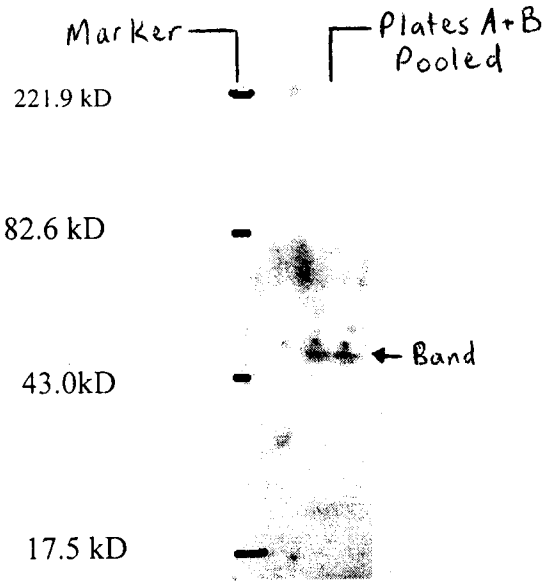


Figure 1: ELISA data showing the reactivity of four antibody-paratuberculosis antigen pairs. Reactivity of the antibody to its antigen was tested from three antigen mixtures. Two antigen mixtures were from a pooled mixture of paratuberculosis antigens (Plate A or Plate B); and one being from *M. paratuberculosis* cell lysate.

Figure 2: The reaction of Ab 28 with its corresponding antigen on a western blot. Ab 28 reacted with an antigen from a mixture of antigens pooled from both Plates A and B. The size of the antigen is around 45kDa.



The reaction of the four selected antibodies was tested in a western blot. The reaction of each antibody was tested against two antigen mixtures; one being pooled antigens from Plates A and B; and the other being *M. paratuberculosis* cell lysate. Thus, unlike in the ELISA, for the western blot Plates A and B were pooled again to form one antigen mixture. The antigens were separated by size before the antibody was added. Ab 11, 28 (Figure 2), and 30 all reacted with an antigen from Plates A and B that was around 45kDa in size. Ab 11 also reacted with the antigen from the *M. paratuberculosis* cell lysate that was around 45kDa. The ideal scenario in the western blot would be to observe that each antibody binds to an antigen of the same size from both antigen mixtures. Ab 11 recognised its target antigen in both antigen mixtures in the western blot, while Abs 28, 30 and 34 recognised their target antigens from the Plate A and B pooled antigen mixtures but not the *M. paratuberculosis* cell lysate. Western blot conditions are harsh on proteins, so the functioning of the antibodies in a western blot shows that they have a stable structure.

Conclusions

Using a novel methodology to simultaneously isolate single chain antibody-paratuberculosis antigen pairs, four antibodies were isolated. It was shown that the antibodies are able to specifically bind to their target antigens when the antigens are produced by *E. coli*, and produced naturally by *M. paratuberculosis*. It was also shown that the antibodies could function in 'foreign' conditions, such as those in a western blot. Ab 11 is an especially promising antibody. It reacted strongly with its target antigen from the *M. paratuberculosis* cell lysate mixture in the ELISA; and it reacted with the antigen from both of the antigen mixtures used in the western blot.