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B-cell epitopes recognized by Chinese water buffaloes (Bos buffelus) on the 22 kDa tegumental membrane-associated antigen (Sj-22) of the Asiatic bloodfluke, Schistosoma japonicum

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Abstract—The 22.6 kDa tegumental membrane-associated antigen of schistosomes is of recognized importance in immunity to schistosomiasis. In China, bovines are known to play an important role in the transmission of Schistosoma japonicum. Ten buffaloes (Bos buffelus) were vaccinated with a recombinant form (reSj-22) of the S. japonicum 22.6 kDa tegumental antigen (Sj-22) and the sera were used to identify and map possible linear B-cell epitopes on this molecule using a series of 18 overlapping synthetic peptides (P1–P18). Sera from all of the ten vaccinated buffaloes reacted strongly with Sj-22 in western blots and in ELISA, while sera from a further ten adjuvant (Quil A) control buffaloes did not. Four peptides (P3, P8, P9 and P10) were predominantly recognized by at least 90% of the buffalo sera. This pattern of recognition is similar to that obtained in a previous study we undertook in mice immunized with the same antigen whereby peptides 3, 8, 9 and 10 were recognized by over 80% of CBA strain mice. The peptide most frequently recognized by mice (peptide 6), and mapping to an EF-hand calcium binding domain, was recognized by six of the ten vaccinated buffaloes. The major difference between buffaloes and mice occurred with peptide 1 which was recognized very frequently by all three strains of mice tested but was only weakly recognized by three of the ten buffaloes. This study provides a valuable reference for further study on the immunity stimulated by the 22.6 kDa tegumental antigen in the murine model and a natural bovine host of Schistosomiasis japonica. © Inra/Elsevier, Paris.

Schistosoma japonicum / recombinant tegumental antigen / B-cell epitope / Bos buffelus / Chinese water buffaloes

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Résumé – Reconnaissance par les buffles domestiques chinois (Bos buffelus) des épitopes des cellules B situés sur l’antigène de 22 kDa associé à la membrane tégumentaire (Sj-22) de la forme sanguine de Schistosoma japonicum. L’importance de l’antigène de 22,6 kDa, associé à la membrane tégumentaire du schistosome, est reconnue dans l’immunité contre la schistosomose. En Chine, on sait que les bovins jouent un rôle important dans la transmission de Schistosoma japonicum. Dix buffles (Bos buffelus) ont été vaccinés avec une forme recombinante (reSj-22) de l’antigène tégumentaire de 22,6 kDa (Sj-22) de S japonicum, et leurs sérums ont été utilisés pour identifier et cartographier les épitopes linéaires possibles des cellules B sur cette molécule, grâce à une série de 18 peptides synthétiques chevauchants (P1-P18). Les sérums des dix buffles vaccinés ont réagi fortement avec Sj-22 en western blot et en Elisa, alors que les sérums provenant de dix autres buffles contrôles ayant reçu l’adjuvant (Quil A) seulement n’ont pas réagi. Quatre peptides (P3, P8, P9 et P10) ont été principalement reconnus, par au moins 90 % des sérums des buffles. Ce profil de reconnaissance est similaire à celui que nous avions obtenu dans une étude précédente chez la souris immunisée par ce même antigène, et où les peptides 3, 8, 9 et 10 étaient reconnus par plus de 80 % des souris CBA. Le peptide le plus fréquemment reconnu par les souris (peptide 6), et cartographiant un domaine en « EF-hand » liant le calcium, a été reconnu par six des dix buffles vaccinés. La différence majeure entre les buffles et les souris concernait le peptide 1, qui était très fréquemment reconnu par les trois souches de souris testées, mais était seulement faiblement reconnu par trois des dix buffles. Cette étude fournit une référence précieuse pour l’étude de l’immunité stimulée par l’antigène tégumentaire de 22,6 kDa, chez le modèle murin et chez le bovin, hôte naturel de la schistosomose japonaise.


Schistosoma japonicum / antigène recombinant tégumentaire / épitope de cellule B / Bos buffelus / buffle domestique Chinois

1. INTRODUCTION

Schistosomiasis japonica remains a significant threat for some 100 million residents and is a major cause of morbidity in 1.58 million people in the endemic areas in China [2]. Unlike other human schistosomes, Schistosoma japonicum occurs as a natural parasite of domestic animals and some other mammalian species, which act as reservoirs for the infection. Based on long-term epidemiological investigations in the lake regions in China, it has been proved that bovines, especially water buffaloes, play major roles in the transmission of the parasite to both humans and domestic animals. Development and application of an effective anti-schistosomiasis vaccine is considered to be one of the major priorities for schistosomiasis control. The 22.6 kDa tegumental membrane-associated antigen (Sj-22) is recognized as an important antigen involved in immunity against schistosomiasis [5, 6]. The 22.6 kDa native and recombinant proteins of the adult Schistosoma mansonii (Sm-22) are recognised by antibodies from mice protectively vaccinated with isolated tegumental surface membranes [1]. Furthermore, the presence of IgE antibodies against Sm-22 is predictive of the resistance to reinfection in humans [7]. We have mapped B-cell epitopes on Sj-22 [5] and have recently shown that the production of murine IgE antibodies against the 22.6 kDa antigen of schistosomes is directed by the antigen itself [6]. We have immunized water buffaloes with a recombinant form of Sj-22 (reSj-22) in China and here we report the results of B-cell epitope mapping of the Sj-22 molecule with the resultant sera.

2. MATERIALS AND METHODS

2.1. Animals and immunization

Twenty, 9-month-old, helminth-free, male buffaloes each weighing approximately 150 kg were purchased from Longhui County (a non-endemic area for schistosomiasis), Hunan, China.
The buffaloes were held in the Hunan Institute of Veterinary Medicine for the duration of the experiment. The 20 buffaloes were randomly divided into two groups. One group was immunized with purified reSj-22 (vaccinated group) [4, 5] and the other acted as an adjuvant control group. All the buffaloes were immunized with equal volumes (2 mL) by subcutaneous injection into the neck and legs. On day 1, the vaccinated group was immunized with 1 mL (1 mg) of reSj-22 mixed with 1 mL (2 mg) of Quil A adjuvant (Superfos, Denmark) while the adjuvant control group was injected with 1 mL of normal saline mixed with 1 mL of Quil A. These initial injections were followed by two sets of booster injections administered at 14 day intervals on day 15 and day 29. The buffaloes were bled for serum from the ear vein prior to the commencement of immunization and on day 43. The sera collected were used in western blot analysis [6] and to map B-cell epitopes on the Sj-22 protein. Each of the buffalo sera (diluted 1:100) taken after the third immunization (day 43) were tested individually in western blot analysis.

2.2. Peptides and peptide synthesis

The synthesis of Sj-22 peptides used for epitope mapping has been previously described [5]. To assist in the optimization of the peptide-ELISA, the peptides were all synthesized with an additional cysteine residue at the N-terminal to enable chemical linking to the ELISA plate.

The sequences of the peptides synthesized were:

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>CMATTEYRLSMEQFIRAFIE</td>
</tr>
<tr>
<td>P2</td>
<td>CMEQFIRAFIEDKDNELID</td>
</tr>
<tr>
<td>P3</td>
<td>CIDKDNNELIDKQEQTLYKCQO</td>
</tr>
<tr>
<td>P4</td>
<td>CKQELTKYCCQNNQMDMKQIDP</td>
</tr>
<tr>
<td>P5</td>
<td>CNQMMDKQIDPWIARFDT DKD</td>
</tr>
<tr>
<td>P6</td>
<td>CWIARFDT DKDGVSEEFRC</td>
</tr>
<tr>
<td>P7</td>
<td>CGKVSEEEFCRGFGGLKVWEVR</td>
</tr>
<tr>
<td>P8</td>
<td>CGFGLKVWEVRREKEELKRDK</td>
</tr>
<tr>
<td>P9</td>
<td>CREKEELKRDKEGVSTPLLD</td>
</tr>
<tr>
<td>P10</td>
<td>CEGKVSTLPLDIQIAATMSK</td>
</tr>
<tr>
<td>P11</td>
<td>CIIAEDSMYKVKYINCKF</td>
</tr>
<tr>
<td>P12</td>
<td>CAKQYINICCKFKELLDKTSRT</td>
</tr>
<tr>
<td>P13</td>
<td>CKEELDKTSRTGDEVRALAND</td>
</tr>
<tr>
<td>P14</td>
<td>CGDEYRALANDLCAKFLDSEYG</td>
</tr>
<tr>
<td>P15</td>
<td>CLKAFLDSEYGRWQQVIIITG</td>
</tr>
<tr>
<td>P16</td>
<td>CRWWQVIILTGSYWMNFSHEP</td>
</tr>
</tbody>
</table>

2.3. Peptide ELISA

The protocol for the ELISA essentially followed that described by Waine et al. [5]. In brief, peptides were diluted to 10 μg/mL in 0.05 M carbonate coating buffer (pH 9.6), and absorbed onto standard 96 well microtitre plates (polyvinylchloride TiterTek immunoassay-plates (cat. no. 77-173-05 activated; Flow laboratories, the Netherlands) (100 μL/well at 37 °C for 2 h). Following absorption, unsaturated protein binding sites were blocked (150 μL/well) using 3 % polyvinyl pyrrolidone in phosphate buffered saline/0.05 % Tween-20 (PBST) at 37 °C for 1.5 h. The plates were then washed three times with PBST buffer. Buffalo sera were diluted 1:100 in PBST containing 0.3 % polyvinyl pyrrolidone and added to the plates at 37 °C for 1.5 h. The plates were then washed again, and horse-radish peroxidase-labelled rabbit anti-bovine IgG conjugate (diluted 1:5 000 in PBST) was added and the plates incubated for 1.5 h at 37 °C. After further washing, the colour reaction was initiated using O-phenylenediamine dihydrochloride (Sigma). The optical density (OD) was measured at 450 nm in an automated ELISA plate reader. All assays were performed in duplicate. For analysis, OD values were divided into three groups representing negative (OD_{450} < 0.3), positive (OD_{450} 0.3–0.6) and strong positive (OD_{450} > 0.6) responses.

3. RESULTS

In western blot analysis all sera from the reSj-22 vaccinated animals reacted specifically with a 22.6 kDa antigen when either reSj-22 or a soluble adult worm protein extract (SWAP) was used as the antigen source (data not shown). The sera were also tested individually with reSj-22 and with each of the 18 synthetic peptides by ELISA. All of the ten buffaloes immunized with the recombinant antigen reacted strongly with the recombinant antigen (mean OD_{450} = 0.99) and there was a significant difference (P < 0.01) compared with the adjuvant control group (mean OD_{450} = 0.36).
The pattern of peptide recognition by the antibodies from the reSj-22 vaccinated group compared with the control group is shown in figure 1. The recognition of each peptide and the magnitude of response by individual buffaloes is shown in figure 2. Some of the peptides were recognized more frequently and strongly than others but there was no individual buffalo having the same pattern of recognition as any other. Buffalo number 14 (B14) responded most strongly both in terms of peptide number and the magnitude of the reaction. Most of the buffaloes had a strong positive reaction to some of the peptides but three buffaloes (B8, B13 and B20) did not react strongly to any of the 18 peptides.

The most frequently and strongly recognized peptide was peptide 9 which was recognized by all of the ten buffaloes, and seven of the ten reacted strongly with it. Peptide 3 was also recognized by all ten buffaloes and six of the ten reacted strongly with it. Peptide 8 was recognized by nine buffaloes and five of the ten reacted strongly with it. Peptide 10 was also recognized by nine buffaloes but only one reacted strongly with it. The magnitude of the responses to each of the peptide is listed in the order: P9 > P3 > P8 > P10 > P14 > P6 > P13. Six peptides (P1, P2, P5, P7, P16 and P17) were recognized by less than 50% (five) of the buffaloes. The remaining five peptides (P4, P11, P12, P15 and P18) were not recognized by any of the buffaloes.
4. DISCUSSION

The 22.6 kDa tegumental membrane-associated antigen is considered as a potential vaccine candidate against schistosomiasis [3]. The antigen can stimulate production of specific IgG and IgE antibodies [6]. The reSj-22 has been expressed and purified [4] and the B-cell epitopes on this antigen have been mapped using mouse sera [5]. In this paper, we report the identity of B-cell epitopes on Sj-22 using buffalo sera immunized by the recombinant antigen. The results are basically consistent with those obtained with mice [5]. The main peptides (P3, P8, P9 and P10) recognized by the sera from both buffaloes and mice are located in the hydrophilic N-terminal half of the molecule. Peptides 15 and 18 were not recognized by buffaloes or mice. Peptides 4, 11 and 12 were not recognized by any of the buffaloes and were recognized by very few mice, with a very weak response. Peptide 13 was recognized weakly by buffalo sera and was not recognized by BALB/c mice but was recognized strongly by CBA mice. The major difference between buffaloes and mice occurred with peptide 1 which was recognized very frequently by all three strains of mice but was only weakly recognized by three of the ten buffaloes. Peptides 8, 9 and 10 were the main peptides recognized by buffaloes and by over 50% of the mice; as they are three consecutive peptides, the dominant antigenic region of Sj-22 may be located in this region. The peptide most frequently recognized by mice (peptide 6), and mapping to an EF-hand calcium binding domain, was recognized by six of the ten vaccinated buffaloes.

![Figure 2](image.png)

**Figure 2.** The pattern of antibody recognition to the Sj-22 peptides determined by ELISA. Peptides are numbered from 1 to 18 and the recombinant antigen is indicated as Teg. Letter B indicates buffaloes (all belonged to the Sj-22 vaccinated group) and the numbers following the letter B indicate individual animals. The magnitude of the responses (OD$_{450}$) is indicated by the shading of squares: strong positive (OD$_{450}$ > 0.6) are black, positive (OD$_{450}$ = 0.3–0.6) are grey and negative (OD$_{450}$ < 0.3) are white. The percentage of animals responding to each peptide is shown on the bottom line. All vaccinated animals responded to the reSj-22 m ELISA as indicated in the far right hand column.
Overall, the results clearly indicate that the Sj-22 kDa antigen and some of the synthetic peptides could be recognized by antibodies generated in water buffaloes vaccinated with purified reSj-22. There were some differences in the responses of the two animal species to the same antigen and peptides. Nevertheless, these results provide a valuable reference for further study on the immunity stimulated by the 22 kDa tegumental antigen in the murine model and a natural bovine host of schistosomiasis japonica.

ACKNOWLEDGEMENTS

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