

# MONOCLONAL ANTI-IDIOTYPE ANTIBODY TO HSV-1 NEUTRALIZING MONOCLONAL ANTIBODY: PRODUCTION AND CHARACTERIZATION

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## SUMMARY

This study is an attempt to produce and characterize murine monoclonal antibodies directed against the paratope of HSV-1 neutralizing monoclonal antibody. Monoclonal antibody 138 C5G10 which was neutralising and directed to 120 K antigen gB of HSV-1 was used as the idiotypic. We were able to produce three Ab-2 monoclonal antibodies as characterized using immunofluorescence, ELISA and RIA. The findings of the present study suggest that two anti-idiotypes 3AiB3E10 and 3AiB5D10 share the same unique fine specificity while 3AiB3C9 has a different specificity on 138 C5G10 paratope. The utility of such 'surrogate' antigens in serological assays and modulation of immune response is discussed.

KEY WORDS Monoclonal antibody Anti-idiotype Herpes simplex virus

ABBREVIATIONS Ab-1: Antibody 1 Ab-2: Antibody 2 ELISA: Enzyme Linked Immunosorbant Assay RIA: Radio Immuno Assay NMS: Normal mouse serum

## INTRODUCTION

Easy transmission from infected to susceptible hosts, latency, periodic reactivation of virus, and virus excretion associated with reactivation, are the chief characteristics of *herpes simplex virus* (HSV) infection. In view of the fact that immunity to a single major glycoprotein is adequate for neutralizing the virus, anti-idiotypes may function as surrogate antigen. Such an anti-idiotype would represent the internal image and mimic the antigens of the infectious virus to a susceptible host (Dreesman and Kennedy, 1985). Further, as a consequence of antigenic cross-reactivity between HSV-1 and HSV-2, serological detection of specific antibody against either of the serotypes of the herpes simplex virus is difficult. To circumvent this problem an anti-idiotype 'mirroring' the type-specific epitope universally present in all isolates of that particular serotype may be used to identify the infection.

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## MATERIALS AND METHODS

*Specificity of HSV hyperimmune sera raised in rabbits*

HSV-1 antigen was made in primary rabbit kidney cells (Lennette and Smith, 1979). This antigen was used for raising hyperimmune sera in rabbits of Haffkein strain. Titration of the hyperimmune sera was done by indirect hemagglutination (Bernstein and Stewart, 1971) with modifications (Seth *et al.*, 1978). The relative reactivity of serum to HSV-1 and HSV-2 antigen was expressed as II/I index (Prakash and Seth, 1979). An index value of < 85 was taken as an indication of HSV-1 specific response while a value of > 85 was considered as HSV-2 specific antibody response (Seth *et al.*, 1978).

*Immunization for producing anti-idiotypes*

## Immunizing Idiotype:

Mouse monoclonal antibody 138 C5G10 (gifted by Dr P Rajamouli) which was raised and characterized in this laboratory was used for immunization. This antibody (Ab-1) reacted only with HSV-1 and not with HSV-2 infected vero cells in ELISA (Enzyme Linked Immunosorbant Assay) (Frame *et al.*, 1984) and immunofluorescence. In radio-immuno precipitation assay (Pereira *et al.*, 1980) it precipitated a 120 kilodalton glycoprotein corresponding to glycoprotein B of HSV-1 (gB-1) from infected vero cell lysates of HSV-1 only. It did not precipitate any protein from HSV-2 infected vero cell lysates. This antibody was tested for neutralising activity against AC strain (Gupta *et al.*, 1972) of HSV-1 and 219 strain (Seth *et al.*, 1974) HSV-2 by plaque reduction assay (Figueroa and Rawls., 1969). The results of this test showed that a plaque reduction of 50% was obtained for HSV-1 by this antibody using culture supernatant.

## Immunization Schedule:

Six-week old inbred BALB/c mice were immunized according to the following schedule:

Dose	Route	Day
200 µg(Ab-1)+CFA 100 µg(Ab-1) in saline	ip sc	0
300 µg(Ab-1) + CFA	ip	7
300 µg(Ab-1) + CFA	ip	14
300 µg(AB-1) in saline	iv	24

Three days after the last dose the mice were sacrificed and the spleens collected aseptically for cell fusion (Kohler and Milstein, 1975).

*Cell Fusion*

The splenic lymphocytes were fused with SP2/0 myeloma cells in 1:1 ratio in the presence of polyethylene glycol 4000. The cells were suspended in a selective medium consisting of RPMI 1640 supplemented with 10% FCS, hypoxanthine, aminopterin and

thymidine at a concentration of  $5 \times 10^5$  spleen cells/ml. 1 ml of this suspension was added to every well of 24 well tissue culture plate (Nunc, USA) which already contained a feeder layer of mouse peritoneal macrophages at a concentration of  $1 \times 10^5$  cells/ml. They were incubated at  $37^\circ\text{C}$  in presence of 5%  $\text{CO}_2$ . Supernatants from wells containing growing hybrids were screened for the presence of Ab-2 (antibody-2). Those found positive were cloned and further subcloned by limiting dilution to ensure monoclonality. They were screened at every stage.

### *Screening for Ab-2*

#### Immunofluorescence:

Surface immunofluorescence staining was done on cyto centrifuge smears (Thanavala *et al.*, 1985a). Cells secreting Ab-2 clones were washed thrice in PBS, pH 7.4 in cold and cell concentration was adjusted to  $1 \times 10^6$  cells/ml in PBS, pH 7.4. Smears of  $100\mu\text{l}$  of the cell suspension were made by spinning in cyto centrifuge (Shandon, cytospin 2) at 700 rpm for 10 min. The smears were air dried for 10 min at room temperature and fixed at  $4^\circ\text{C}$  for 20 min in a mixture of cold 95% ethanol and 5% acetic acid. For staining, the smears were incubated for 1 h at room temperature with either normal rabbit serum or anti HSV hyper immune serum (1:10,000) followed by three washes in cold PBS, pH 7.4 for 10 min each and further incubated with 1:20 dilution of fluoresceinated anti-rabbit serum (Dakopatts) absorbed with mouse spleen and liver acetone extracted powder (Sigma) at 100 mg/ml of neat sera, for 20 min at room temperature. The slides were washed three times in PBS, mounted in buffered glycerol (PBS pH 7.4 and glycerol in ratio of 1:1) and examined under a fluorescence microscope (Carl Zeiss, E. Germany).

#### ELISA:

$2\mu\text{g}$ /well antimouse immunoglobulin serum raised in rabbit was coated on to the polyvinyl immuno plates in carbonate-bicarbonate buffer, pH 9.5, overnight at  $4^\circ\text{C}$ . Non-specific sites were blocked with 1% BSA + 0.5% NMS (normal mouse serum) in PBS, 7.4 for 30 min at room temperature, followed by washings for 3 min each in PBS + 0.025% Tween-20.  $200\mu\text{l}$  supernatant from wells showing cell growth was added to each well and incubated at  $37^\circ\text{C}$  for 2 h, followed by washing as mentioned earlier. Anti HSV-HRP conjugate was then added to the wells and further incubated at  $37^\circ\text{C}$  for 2 h, followed again by washing. Substrate O-phenylene diamine (OPD) in acetate buffer, pH 5.0 was added to each well and left for color development in the dark at room temperature. The reaction was stopped by adding  $25\mu\text{l}$  of 2M  $\text{H}_2\text{SO}_4$  and O.D. was read at 492 nm in the ELISA reader.

### *Characterization of Ab-2*

#### Solid phase RIA (Radio Immuno Assay): Ab-2 Binding Specificity:

Polyvinyl immunoplates were coated with monoclonal antibody 138 C5G10 (Ab-1) and also with a panel of 7 irrelevant monoclonal antibodies in PBS, pH 7.4 at  $4^\circ\text{C}$  for 18 h. Wells were then washed with PBS three times and blocked with PBS containing 1% BSA and 0.5% normal mouse serum for 30 min at room temperature followed again by washing with PBS containing .05% Tween-20. 20,000 counts of each radioiodinated monoclonal Ab-2 were added in triplicate. The plates were left for incubation at  $4^\circ\text{C}$  for

4 h. The wells were washed again and air dried. Individual wells were cut and gamma counted. Results were expressed as percentage binding. Binding to myeloma supernatant served as negative control.

#### Solid Phase RIA: Inhibition of Antigen Binding by Ab-2:

Polyvinyl immunoplates were coated with 138 C5G10 (Ab-1) and subsequently blocked and washed as described above. .1, 1, 10 and 100 ng of Ab-2 was added in triplicate at 4°C for 1 h. gB-1 was affinity purified using 138 C5G10, radioidinated (Reddy *et al.*, 1977) and 50,000 counts of radioiodinated immunoaffinity purified gB-1 (calculated as optimal binding activity with 138 C5G10) were also added. The positive control comprised the serum from the same animal that was immunized with 138 C5G10 to raise these Ab-2 clones. Normal mouse serum served as the negative control of inhibition. The result is seen as a decrease in cpm bound of radiolabelled gB-1 with increase in Ab-2 concentration. Inhibition of total binding was calculated as follows:

$$\text{Total binding} = \frac{\text{Mean bound cpm (Ab-2 combinations)} \times 100}{\text{Absolute binding (gB-1* + NMS)}}$$

\* Radiolabelled gB-1

Binding inhibition (%) = 100 – Total binding

#### Solid Phase RIA: Ab-2 Fine Specificity:

Polyvinyl immunoplates were coated, blocked and washed as described. Various combinations (Table 2) of fixed counts of radioiodinated and unlabelled Ab-2 (concentration of each Ab-2 which was capable of reducing the negative control radioactivity value in the antigen binding inhibition assay in half) were added in triplicate to 138 C5G10 coated wells. This was incubated for 4 h at 4°C, washed and air dried. Individual wells were cut and gamma counted.

## RESULTS AND DISCUSSION

### *Detection of Ab-2*

Of 8 positive hybrids detected in immunofluorescence and ELISA, three clones 3Ai B5D10, 3Ai B3E10 and 3Ai B3C9 (Figure 1a, 1b and 1c and Figure 2) were stabilized. They showed good reactivity in ELISA and were also found to be secreting Ab-2 when the surface bound Ab-2 was stained by anti-HSV hyperimmune sera in immunofluorescence. It is a possibility that Ab-2 is reacting with interspecies determinants which are not really a part of the actual combining site in molecular contact with HSV antigen but are essential for the construction of binding site of the right specificity (Thanavala *et al.*, 1985b; Urbain *et al.*, 1980; Ju *et al.*, 1981). However, the negative controls consisting of staining of surface bound Ab-2 with rabbit preimmune serum show no fluorescence with any of the three clones.

### *Recognition of Paratope*

Specificity studies were done in solid phase RIA. Each Ab-2 was radioiodinated. Direct binding of radiolabelled Ab-2 to the idiotype 138 C5G10 and 7 other irrelevant

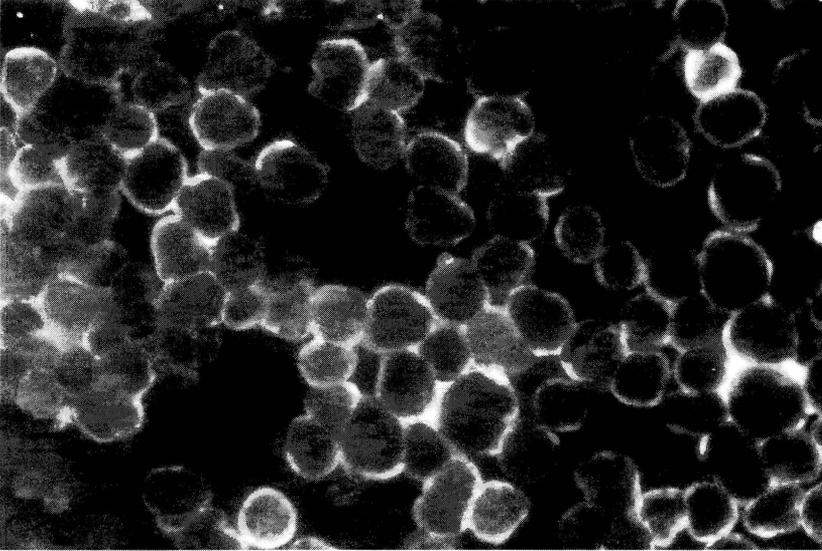


Fig. 1a

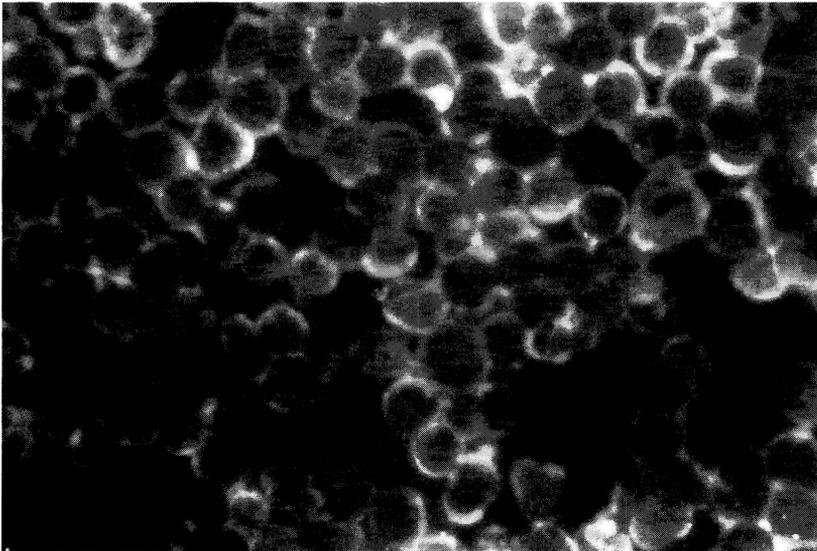


Fig. 1b

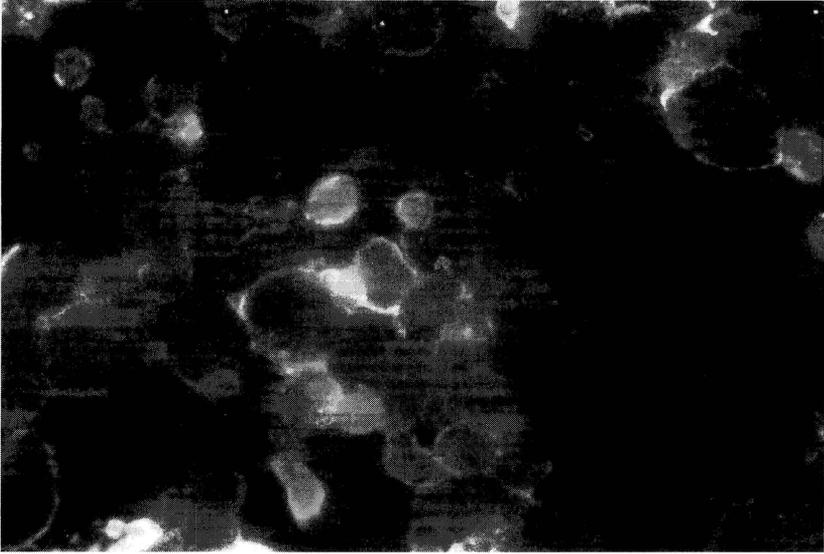


Fig. 1c

Fig. 1. Immunofluorescent staining of AB-2 producing clones. Photomicrographs B showing cytocentrifuge smears of clones (a) 3 Ai B3E10 (b) 3 Ai B5C9 and (c) 3 Ai B5D10 were made. Surface bound Ab-2 was detected by staining with rabbit anti-HSV hyperimmune sera followed by fluoresceinated anti-rabbit serum.

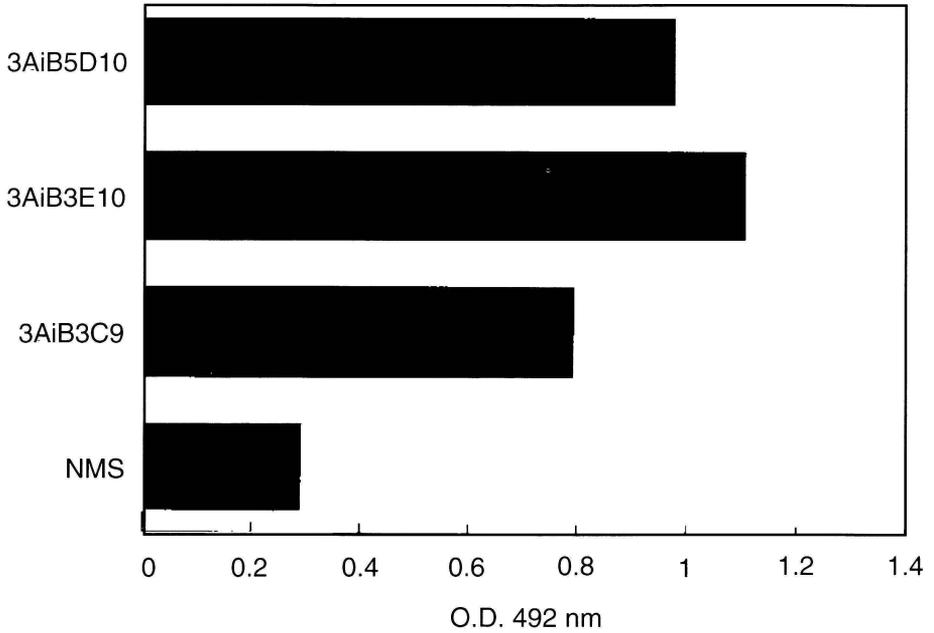


Fig. 2. Screening for Ab-2 by ELISA. Optical density values obtained using culture supernatants of the three monoclonal antibody secreting Ab-2 clones and normal mouse serum (NMS) to EIA plates coated with anti-mouse immunoglobulin serum. Bound specific Ab-2 was detected using anti HSV-HRP conjugate.

monoclonal antibodies (Table 1) were studied. As seen in Figure 3, percentage binding of antibodies 3Ai B3E10 and 3Ai B5D10 to 138 C5G10 was 72.45% and 73.61% respectively. Whereas, the binding of these two Ab-2 monoclonal antibodies to the panel of irrelevant antibodies range from 11.58% to 31.1% and 10.03% to 14.06% respectively. 3Ai B3C9 showed an altogether different binding pattern. The percentage of specific binding was 66.97%. It varied from 10.26% to 47.7% with irrelevant idiotypes. K4 and the three other anti-HSV-1 monoclonal antibodies (137 C2C5, 137 C5F12 and 137 C2C8) exhibit highest reactivity to 3Ai B3C9. The specificities of these four 'irrelevant' antibodies do not fall into any one category. 137 C2C5 and 137 C2C8 share specificities and compete with each other but not with 138 C5G10. On the other hand 137 C5F12 reacts specifically to gE-1, while the specificity of K4 is unknown although it is certain that the antibody was not raised against either HSV-1 or HSV-2. The only common feature is that all of them are of murine origin. This suggests that 3Ai B3C9 may be identifying a region which is very close to the binding site of 138 C5G10 and which is not unique to it but is shared by other related and unrelated mouse monoclonal antibodies. The concept of internal image does not imply that the Ab-2 molecules carry a structure resembling the entire antigenic site. Thus, an individual Ab-2 beta molecule would react only with those antibodies that use a particular set of contact residues to bind to the antigen. Since all Ab-2 that are induced are unlikely to be Ab-2 beta type but include antibodies directed against a framework idio type of Ab-1 that tends to be found in association with paratopes of a particular specificity (Roitt *et al.*, 1986).

Table 1. Specificities of Panel of Monoclonal Antibodies used for Testing Ab-2 Specificity

	Antibody	Specificity
1.	138 C5G10	HSV-1 (gB-1)
2.	137 C2C5	HSV-1 (gB-1)
3.	137 C5F12	HSV-1 (gE-1)
4.	137 C2C8	HSV-1 (gB-1)
5.	BB	HSV-2
6.	K1	Unknown
7.	K4	Unknown
8.	K8	Unknown
9.	Myeloma	Unknown

#### *Ab-2 mimics epitope of antigen*

To determine whether all the three Ab-2 bore the internal image of the original gB-1, their ability to block binding of radiolabelled antigen to 138 C5G10 was examined (Figure 4). 3Ai B5D10 and 3Ai B3E10 were able to inhibit the binding of radioiodinated gB-1. However, 3Ai B3C9 was less efficient in this assay. 3Ai B5D10 and 3Ai B3E10 reduced the radioiodinated antigen binding to 18.02% and 21.97% respectively while 3Ai B3C9 reduced the binding to nearly 30%. This demonstrates that these Ab-2 were not only reacting with the paratope of 138 C5G10 but may also be mimicking an epitope found on gB-1. Therefore, these Ab-2 inhibited the subsequent reaction of 138 C5G10

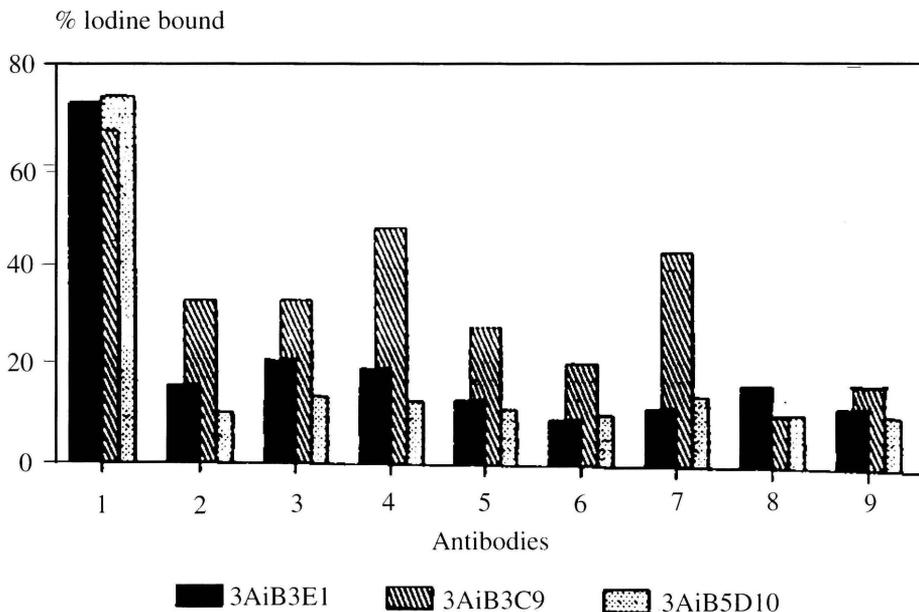


Fig. 3. RIA for Ab-2 specificity. Antibodies 1, 2, 3, 4, 5, 6, 7, 8, and 9 denote antibodies 138 C5G10, 137 C2C7, 137 C5F12, 138 C2C8, B3, K1, K4, K18 and Myeloma supernatant respectively which were used for coating microwells. To these was added 20,000 counts of <sup>125</sup>I labelled Ab-2 in triplicate. Readings are represented as an average mean.

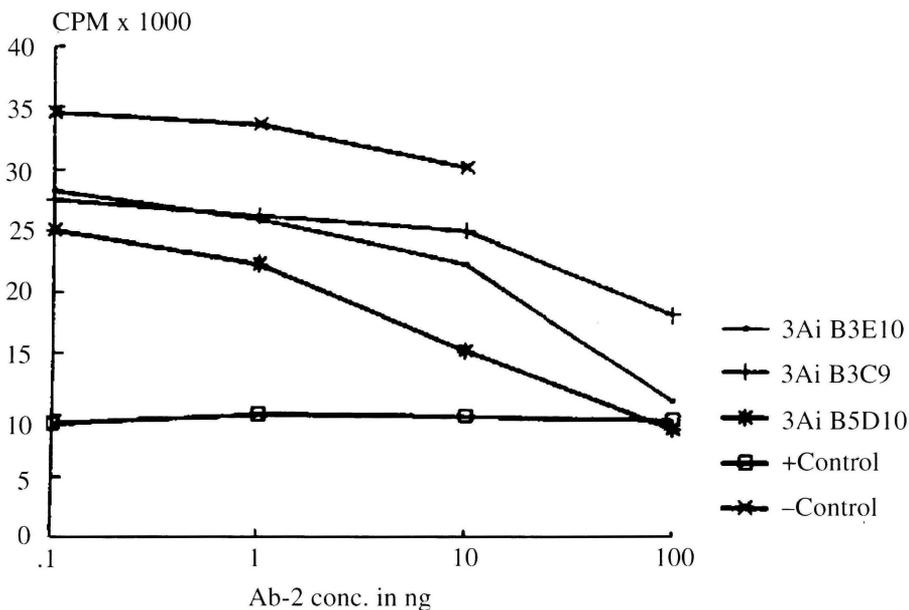


Fig. 4. Blocking of antigen binding by Ab-2 in RIA. Binding curves of Ab-1 to Ab-2, mouse hyperimmune serum and normal mouse serum.

with antigen. Hence, both Ab-2 and gB-1 bind to the same paratope of 138 C5G10 or a region very close to the paratope.

#### *Fine specificity of Ab-2*

Fine specificities of binding of Ab-2 to 138 C5G10 were determined by putting combinations of Ab-2, one radiolabelled and the other not radiolabelled, in competition. Table 2 shows that 3Ai B3E10 and 3Ai B5D10 inhibited the binding of each other to 138 C5G10. 3Ai B3E10 inhibited total binding of radioiodinated 3Ai B5D10 by 66.8% and 3Ai B5D10 inhibited the total binding of radioiodinated 3Ai B3E10 by 83.2%. On the other hand 3Ai B3C9 inhibited the total binding of 3Ai B3E10 by 30.9% and that of 3Ai B5D10 by 5%. This suggests that 3Ai B3E10 and 3Ai B5D10 shared the same unique fine specificity while 3Ai B3C9 shares a different specificity on 138 C5G10 paratope.

Table 2. Fine Specificity of Binding of Ab-2

Combinations of Ab-2	Counts added (cpm)	Mean <sup>125</sup> I bound (cpm)	Total binding inhibition (%)
1*+2	20,000	9920	30.9
1*+3	20,000	2418	83.2
2*+1	20,000	3607	65.82
2*+3	20,000	2042	80.71
3*+1	20,000	4562	66.81
3*+2	20,000	13049	5.05
1*+NMS	20,000	14355	
2*+NMS	20,000	10551	
3*+NMS	20,000	13743	

- i) 1\*, 2\* and 3\* represent radiolabelled Ab-2 antibodies 3AiB3E10, 3AiB3C9 and 3AiB5D10 respectively.  
 ii) 1, 2 and 3 represent unlabelled Ab-2 antibodies 3AiB3E10 and 3AiB3C9 and 3AiB5D10 respectively.

The diagnostic value of serological assays is limited by multiple antigens shared by HSV-1 and HSV-2. There may also be some heterotypic anamnestic responses to varicella-zoster virus in persons infected with HSV and vice versa. Added to this, the purification of specific antigens of HSV-1 or HSV-2 is a tedious process. With the use of anti-idiotype that represents a specific antigen of HSV-1 or HSV-2 and its role in trapping type specific antibodies the serological assays will be more meaningful.

It has been shown in many systems that anti-idiotypic reagents that mimic epitopes on appropriate antigens can be substituted for antigen-specific immune response *in vivo* (Kennedy and Dreesman, 1985). Modulation of Herpes simplex virus infection is of great interest (Kennedy *et al.*, 1984; Gell and Moss, 1985; Lathey *et al.*, 1986). Kennedy and his group (Kennedy *et al.*, 1984) reported that polyclonal anti-idiotype reagents increased the pathogenicity of HSV-2 infection on challenge after anti-idiotype administration. In contrast to this, another study (Gell and Moss, 1985) reports that polyclonal

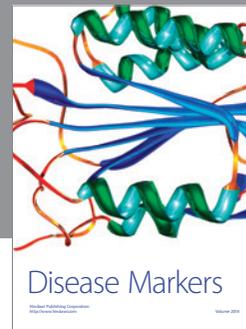
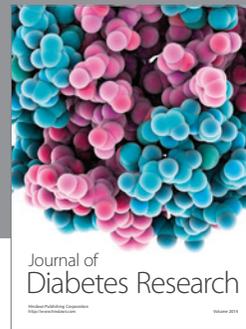
rabbit anti-idiotypes could generate dose dependent delayed type hypersensitivity responses when mice were challenged with HSV-1 subcutaneously in the ear pinna after prior administration of anti-idiotype serum. Immunization of mice with anti-idiotype to glycoprotein D of HSV was seen to prime mouse splenocytes *in vivo* to proliferate in response to HSV antigen stimulation *in vitro* (Lathey *et al.*, 1986).

The paratope-idiotypic interactions between B cells and regulatory T cells, have a physiological regulatory effect. There exist regulatory T cell counterparts for Ab-1 and Ab-2 antibodies (Bona, 1981). Therefore, we are now in the process of testing the functional efficacy of our Ab-2 and their role in inducing cell mediated immunity. We have tried to assess the antigen specific priming ability of the Ab-2 antibodies by immunizing mice with various doses of each Ab-2 in different sets and subsequently stimulating the T cells with affinity purified gB-1 antigen in culture. Preliminary results show that two out of three Ab-2, 3Ai B3E10 and 3Ai B5D10 are able to prime the mouse T cells *in vivo*. These Ab-2 primed cells were able to recognise the *in vitro* stimulation signal of the antigen gB-1 and were consequently seen to proliferate as assessed by incorporation of tritium labelled thymidine from the culture medium. The stimulation indices obtained were comparable to those obtained in the positive control for proliferation where the mice T cells were both primed and stimulated with affinity purified gB-1 at optimally calculated doses. This pilot study reveals a very interesting aspect of the characteristics of these Ab-2 which has importance in determining their value as surrogate antigens in the construction of subunit vaccine.

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