Production of Cell-mediated Immune Response to Herpes Simplex Virus by Immunization with Anti-idiotypic Heteroantisera

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SUMMARY

Three BALB/c monoclonal antibodies capable of neutralizing herpes simplex virus type 1 (HSV-1) were used to prepare rabbit anti-idiotypic antisera. Affinity-purified antibodies from four of these rabbits were used to immunize mice by repeated subcutaneous injection over a period of 6 to 7 weeks: the mice were then challenged with HSV-1 subcutaneously in the ear pinna. Measurement of ear swelling showed that prior administration of anti-idiotypic serum could generate dose-dependent delayed-type hypersensitivity responses.

It is recognized that anti-idiotypic (anti) antibodies can be directed in some instances against part or all of the combining sites of the antibodies concerned, and that such antibodies make a more or less accurate 'model' of the epitope stimulating the original antibody (see Bona, 1981). When the immunogen is a virus (or other infectious agent), the anti-idiotype when used as an immunogen may therefore stimulate a response similar to that against a component of the original virus. In this paper we study this phenomenon as illustrated by cell-mediated responses to herpes simplex virus type 1 (HSV-1) in mice. The anti-idiotypic antibodies were all raised in rabbits and directed against monoclonal antibodies reacting with glycoproteins of HSV-1.

The monoclonal antibodies were those described by Maclean-Pieper (1982) and raised in BALB/c mice. Rabbit antisera were made against three of these, LP 2 (two rabbits), LP 11 and LP 14. LP 2 and LP 14 were directed against the type-common glycoprotein D (gD) of HSV-2, strain BRY. They showed complete immunological cross-reactivity and cross-neutralization with the gD of HSV-1 strain Sc 16. This strain has been used in the work described below since it is the one used routinely in this laboratory for immunological work and we have found that it gives a brisker ear response than our routine strain (BRY) of HSV-2. LP 11 is directed against a less well-characterized glycoprotein (gH) specific to HSV-1 (strain Sc 16). All these were of the IgG2a immunoglobulin subtype. Total immunoglobulin preparations (Ig) were made from these by precipitation at two-fifths ammonium sulphate saturation followed by dialysis and used for immunization of rabbits and for coating of red cells. Rabbits were immunized by two intramuscular injections of 0.5 to 1.0 mg of pepsin-digested Ig in Freund's complete adjuvant given 4 weeks apart, followed by bleeding after 1 further week. Further bleeds were taken after a course of four to six intravenous injections of 0.5 to 1.0 mg of aluminium phosphate-precipitated Ig. No significant differences were noted between the products of the various bleeds.

For titration of the anti-idiotypic antibodies, trypsinized sheep red blood cells (SRBC) were treated with 1 mg of the monoclonal Ig in saline plus 0.05 ml of distilled glutaraldehyde (TAAB Laboratories, Reading, U.K.) with shaking; after 5 min this was made up to 20 ml with saline, the cells were washed by gentle centrifugation several times, and finally made up to 20 ml with normal mouse serum diluted 1/200 in saline for storage. Agglutination reactions using an appropriate dilution of these coupled cells were carried out in plastic trays with round-bottomed wells (Linbro) in 1/200 normal mouse serum/saline. The rabbit sera all showed antibodies against normal mouse serum proteins; these could be readily adsorbed with 1/30 vol. of normal mouse serum, with glutaraldehyde-polymerized mouse serum, or with polymerized monoclonal...
Fig. 1. Mean daily ear swelling increments over normal in groups of six mice immunized with absorbed anti-LP 2 eluate I. Unit doses were given five times at weekly intervals. Unit doses were: A, 18 µg anti-idiotype; B₁, 6 µg anti-idiotype; B₂, 2 µg anti-idiotype; C, 0.6 µg anti-idiotype; C₀₁, 2 µg rabbit gamma globulin; C₀₂, control group (no treatment). Test virus dose given 1 week later was 10 p.f.u. HSV-1 intradermally in left ear pinna with ‘recall’ injections of 10 p.f.u. into right ear on day 18. Swelling increments over normal were recorded on days 1 and 2 after injection.

 ascitic fluid of a different specificity. After absorption, the sera were negative at 1/10 with heterospecific coated SRBC and gave specific agglutination, with a satisfactory preparation of SRBC, to a titre of 1:50000 or better. For affinity purification of antibody, specific immunosorbsents were made by attaching purified monoclonal antibody Ig to CNBr-activated Sepharose at 1 mg per g. After washing of the gel, antibody Ig or crude antiserum was added; after 10 min shaking the deposit was thoroughly washed with saline, and the absorbed antibody eluted with 0.1 M-glycine-HCl pH 2.8. The eluate was rapidly neutralized with solid disodium tetraborate and dialysed against saline. Eluted antibody was usually found to be at a protein concentration of 0.1 to 1.0 mg/ml.

BALB/c mice were immunized in groups of six. Five deep intradermal injections were given at weekly intervals. Mice were tested by intradermal injections of virus into the ear pinnae, and the subsequent ear swelling was measured over the next 5 days with a Mitutoyo engineer's micrometer.

The ‘recall’ injections were made similarly in the other ear; doses etc. are given in the legends of Fig. 1 and 2. Statistical comparisons were made between groups by means of Wilcoxon ranking tests; that is, the probability that both groups come from the same population is estimated (see Colquhoun, 1971).

Fig. 1 illustrates the results with an eluate from serum R II raised against LP 2. Five groups of six mice were sensitized as described above, using five weekly doses of 0.6, 2.0, 6.0 or 18.0 µg of the affinity-purified eluate of anti-LP 2. The control groups received either 2.0 µg of normal rabbit Ig or no treatment. One week after the end of this injection course, all the mice were tested by injection into the left ear pinna of 10 p.f.u. HSV-1 (Sc 16) in 0.01 ml (day 0) and ear thickness was measured daily up to day 4, three mice in each group having the ear removed after day 3 for
Short communication

Fig. 2. Ear swelling increments over normal in mice immunized with absorbed anti-LP 2 eluate 2 (a), anti-LP 11 eluate (b), anti-LP 14 eluate (c) or a mixture of all three (d). Techniques as in Fig. 1. Unit doses were: A, 0.4 μg; B, 2.0 μg; C, 10 μg; Co, 2.0 μg normal rabbit Ig.

virus titration. The results plotted are the measurements after subtraction of the mean thickness of the normal, untreated ears; they are the means of six mice in each group (apart from day 4). There was a marked peak in two of the test groups (B and C) at the second day whereas the control groups (Co₁ and Co₂) started low and continued to rise; the group that received normal rabbit Ig was indistinguishable from that which received no treatment. Using a Wilcoxon ranking test, the probability that each of the test groups at this day came from the same population as the combined control groups was <0.01 in each case; the differences between these and test group A which received the largest dose were not significant. On days 4 and 5 there were significant (<0.01) depressions of the combined test groups (B₁ + B₂) below the level of the combined control groups (Co₁ + Co₂).

The results of the ‘recall’ test of all groups on days 28 + 1 and 28 + 2 illustrate in the control groups what sort of values are to be expected from pre-sensitized mice, and in the ‘test’ groups the absence of any signs at this stage of suppression or induced tolerance resulting from the pretreatment with anti-idiotype; that is to say, the results in all groups were as expected for normal mice after sensitization by the test dose of virus.

Fig. 2 shows the results with eluates corresponding to LP 14, LP 11 and an eluate (2) from serum of another rabbit (R XI) immunized with LP 2. Doses for sensitization were (A) 0.4, (B) 2.0 or (C) 10 μg and (Co) 2.0 μg of rabbit Ig for the control groups. In this case the values shown are those obtained after subtraction of the mean value of a control unimmunized group at day 1. Fig. 2(d) shows the results after immunization with all three eluates simultaneously; the dose of each idiotype was as before, but the total dose reckoned as rabbit Ig was three times as much. Note that the same control group (Co, six mice) is used in all four graphs. Compared with Fig. 1, the peaks in each were 1 day later, and as before showed significances of <0.01 by the Wilcoxon test, apart from the results in Fig. 2(d), which were only marginally significant (<0.05). The aim of this last group was to ascertain whether reactivity to three different determinants would produce markedly more intense responses than that to one alone, but this was clearly not the case.
We conclude that statistically significant differences between immunized and control groups were found at the second or third day after testing, at a time when the maximum response is to be expected in pre-sensitized mice. This was evident in all five experiments recorded using four different anti-idiotypic eluates. This indicates that delayed hypersensitivity has been induced by the anti-idiotype. A positive response of this nature is in contrast to the results of Kennedy et al. (1984) using a HSV system, who found suppressor responses; this is not altogether surprising since they used different antisera in different doses. In our system, the immunizing dose of anti-idiotype has been found to be critical; a change of unit dose from 2 µg to 20 µg largely abolishes any positive effect. In most experiments there was a sharp fall in swelling between day 3 and day 5; although this is often observable after re-testing a normally sensitized mouse, it is possible that some suppressive effect supervenes at this time. Our data are unfortunately not adequate to demonstrate this, if it occurs. In other systems including hapten systems, a positive delayed response has frequently been demonstrated (for references, see Kennedy & Dreesman, 1985).

One experiment was done (not shown) in which 1 to 6 µg doses of the eluates were injected into the ears of mice pre-sensitized with HSV; no positive results were recorded. This is not surprising in view of the polyclonal nature of the anti-HSV response; whether or not our anti-idiotypes have actually modelled epitopes of the virus, there is no reason to expect that the particular epitopes we have used involve a major part of the normal total response of the mouse to HSV.

Although it has been observed (Nash & Gell, 1981) that there is some correlation between delayed hypersensitivity and protection as judged by accelerated clearance of virus from the ear, we were not able to demonstrate this using ears taken at the fourth day from some of the mice used in the above experiments, including that illustrated in Fig. 2(d). However, work along these lines is being pursued. We are also studying the possible 'switch-on' of antibodies bearing the immunizing idiotypes in the serum of the immunized mice.

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**REFERENCES**


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