Epitope Mapping of Monoclonal Antibodies to \textit{gag} Protein p19 of Avian Sarcoma and Leukaemia Viruses

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SUMMARY

We have characterized a set of 15 monoclonal antibodies to p19\textit{gag}, one of the internal proteins of avian sarcoma and leukaemia viruses. All the antibodies work in immune precipitations as well as in immunoblotting, though with different efficiencies. We have developed a simple epitope mapping technique, which uses partial chemical cleavages at methionine or tryptophan residues followed by immunoblotting from SDS-polyacrylamide gels, to localize the epitopes of nine of these antibodies. The epitopes fall into at least four classes. The mapping procedure should also be useful for other antigens of known primary structure.

The avian sarcoma and leukaemia viruses (ASLV) are a group of closely related retroviruses that can induce a variety of tumours in birds. The internal structural proteins of ASLV, known as the \textit{gag} proteins, account for 80\% of the total viral protein. They are synthesized as a single large precursor polypeptide, which is processed proteolytically during virus assembly to yield the five mature proteins p27, p19, p15, p12 and p10 (Weiss \textit{et al.}, 1982), as well as at least two smaller peptides (Pepinsky \textit{et al.}, 1986). In cells transformed by several defective ASLV, \textit{gag} sequences also exist as components of transformation-specific proteins. At least eight distinct viral oncogenes have been found to be fused to the 5' portion of the \textit{gag} gene, thus giving rise to hybrid \textit{gag--onc} proteins.

The N-terminal moiety of the ASLV \textit{gag} precursor is p19, the \textit{gag} protein that interacts with the lipid bilayer in virus particles (Pepinsky & Vogt, 1984), and which presumably plays a key role in organizing virus structure. Because of its ability to bind to RNA \textit{in vitro} (Leis \textit{et al.}, 1978, 1980), p19 may also function in replication, RNA splicing (Leis \textit{et al.}, 1980), packaging of RNA or translation. Monoclonal antibodies (MAbs) to p19 are likely to be of use in studying these several possible functions. Since all ASLV \textit{gag--onc} fusion proteins contain p19, such antibodies also should be of general utility in the study of these proteins. Hybridomas secreting anti-p19 MAbs have been derived in several laboratories (Ingman-Baker \textit{et al.}, 1984; Kurth \textit{et al.}, 1985; Polakova & Russ, 1983) including one in our laboratory at Cornell University and 39 at the University of Pennsylvania. The latter collection was made by fusion of SP2/O-Ag-14 myeloma cells with spleen cells from BALB/c mice immunized with gradient-purified, ether-disrupted avian myeloblastosis virus (AMV). The hybridomas were screened using a solid phase ELISA with purified AMV as antigen. In addition to the anti-p19 hybridomas, at least nine hybridomas secreting anti-p27 MAbs and 11 secreting further unidentified MAbs were isolated.

We have characterized 15 of these MAbs (all IgG1) with respect to approximate location of the epitope on the p19 polypeptide backbone. The method used is based on a CNBr mapping technique described previously (Pepinsky & Vogt, 1984), and may be of general utility in epitope mapping.

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mapping of proteins with few methionine residues, or other residues at which the polypeptide can be specifically cleaved. As applied to p19, the method consisted of the following steps. AMV proteins were separated by electrophoresis on a single wide-well SDS–polyacrylamide gel and a strip containing the p19 band was excised. The gel strip was first incubated with CNBr (which cleaves at methionine residues), yielding a mixture of complete and partial peptide fragments. The gel strip was then laid across the top of a second gel which, following electrophoresis to separate the peptide fragments, was blotted onto a nitrocellulose membrane. Separate parallel strips cut from the membrane were incubated with different MAbs, and the immunoreactive bands were visualized with an enzyme-linked second antibody. The localization of the epitopes on the polypeptide followed immediately from the previously reported CNBr peptide map of p19 (Pepinsky & Vogt, 1984). The same method was applied to peptides generated with N-chlorosuccinimide (NCS), which cleaves at tryptophan residues (Lischwe & Ochs, 1982), thus
Fig. 2. Peptide cleavage sites and epitope locations in p19. The vertical lines below each diagram represent methionine (M) or tryptophan (W) residues found in p19 (Schwartz et al., 1983). The vertical lines above each represent the sites at which cleavages are observed (Pepinsky & Vogt, 1984). The regions to which the epitopes of several of the MAbs have been localized are shown below.

Further defining the location of the epitopes. Analyses were performed on approximately 80 nitrocellulose strips, each containing the peptides derived from approximately 1 μg of intact p19. In SDS-PAGE p19 migrates as a doublet, representing phosphorylated and non-phosphorylated species. The upper band and lower band of the doublet were cut out of the gel separately and processed independently. No differences in antibody reactivity were found between the two.

Fig. 1 shows the profiles of p19 CNBr and NCS peptide fragments recognized by a series of MAbs. Included in this figure are strips that were incubated with a rabbit polyclonal antiserum to p19 (Fig. 1b, lane 1: c, lane 1) to show all the p19-derived peptides that transferred to nitrocellulose and that were available for antibody binding. The composition of these bands is marked at the side of Fig. 1, and the location of the peptides on the p19 backbone is shown in Fig. 2. The NCS bands were not mapped independently, but were assigned to the indicated fragments on the basis of the apparent size of the fragments and the known position of the three tryptophan residues (Schwartz et al., 1983). Some higher mol. wt. aggregates of p19 and its partial peptides also are visible in Fig. 1. Most of these aggregates migrated above intact p19 and had no effect on the analysis. An exception to this is in Fig. 1(c), lane 3 in which two faint bands, with mobilities similar to but distinguishable from bands 2-4 and 3-4, can be seen. We interpret these bands to represent aggregates of smaller NCS peptides of p19.

Some of the MAbs initially produced very weak or undetectable signals by immunoblotting. We investigated several variations in technique to enhance the signal. Incubation with an additional antibody, rabbit anti-mouse IgG, before the enzyme-linked antibody step had little effect. Substitution of an alkaline phosphatase-linked second antibody in place of horseradish peroxidase-linked second antibody led to lower background and thus significantly higher sensitivity. The treatment of the antigen prior to blotting also proved to be important. Virus particles were disrupted with diethyl ether, Triton X-100 or SDS. For the MAbs that had produced strong immunoblotting signals, antigen preparation had little effect. But for MAbs that had produced a weak signal, it was found that disruption with SDS plus 2-mercaptoethanol led to a much stronger signal than disruption by the other two methods. One interpretation of this finding is that disruption by ether or Triton X-100 may lead to the oxidation of sensitive amino acid residues (e.g. methionine), and that some of the MAbs recognize epitopes that include these residues. Whereas initially it had appeared that only half of the MAbs were reactive in immunoblotting, all 15 produced a specific signal when the p19 was prepared by the disruption of virions in SDS plus 2-mercaptoethanol, and an alkaline phosphatase conjugate was used.

From the profiles shown in Fig. 1 and other profiles not shown, it is clear that most of the MAbs fall into two major groups, the properties of which are summarized in Table 1. Members
of group A, comprising MAbs 2D2, 4C11, 4G9, 5C1, 5H9 and 6H12, recognize CNBr peptide 3: the bands corresponding to peptide 3 and all partial cleavage fragments containing fragment 3 (partials) reacted with these antibodies, but the other two bands, corresponding to sequences N-terminal to fragment 3, did not react (e.g. Fig. 1 a, lane 5). Group A MAbs reacted with NCS band 3–4 and larger partials, but not with bands 1, 1–2, 1–3 (e.g. Fig. 1 c, lane 8). Thus the epitope appears to reside between amino acids Trp 97 and Met 139. The detection of reactivity to NCS peptide 4 would strengthen this assignment, but we have been unable to locate this C-terminal peptide on the gels. This may be related to our previous observation in which the C-terminal CNBr peptides of p19 and the p19-related protein p23 were undetectable on SDS–urea gels (Pepinsky & Vogt, 1984).

Members of group B, comprising MAbs 1A1, 1C3, 1G10, 2C10, 2D6 and 6F2 may recognize a more complicated epitope. On the CNBr peptide profile these antibodies bound strongly only to the band corresponding to intact p19 and to the band where CNBr peptides 1–3 and 2–4 comigrate (e.g. Fig. 1 b, lane 6). Although difficult to see in the figure, there was also a weak recognition of CNBr peptides 2–3, 1–2 and 1, but not of peptides 3–4 or 3, thus suggesting that CNBr peptides 1 and 2 contain at least some of the structural features recognized by the antibodies. The epitope(s) for this set of MAbs was susceptible to destruction by ether or Triton X-100, or by NCS, precluding further analysis by NCS cleavage. The pattern of reactivity to the CNBr peptides could be a result of a three-dimensional epitope that includes amino acid residues from peptides 1 and 2.

At least two of the three remaining MAbs appear to have distinct epitopes. The one isolated at Cornell University, 2A7, recognized peptide 2 and all of its partials (Fig. 1 b, lane 5). This corresponds to amino acid residues 41 to 63. NCS treatment destroyed the epitope. Antibody 3C2 also recognized an epitope in the N-terminal portion of p19, but this one was not destroyed by NCS. Bands corresponding to peptide 1 and all its partials reacted with the antibody, limiting the epitope to residues 1 to 51 (Fig. 1 c, lane 3). CNBr peptide 1–2 (residues 1 to 63) reacted strongly, but peptides 1 (residues 1 to 40) and 2–3 (residues 41 to 139) did not (Fig. 1 a, lane 2), suggesting that the epitope may contain the CNBr cleavage site between peptides 1 and 2. The
last antibody, 6H9, gave a CNBr peptide profile (Fig. 1a, lane 7) similar to the profile of class B MAbs. However, the epitope for this antibody was not destroyed by NCS treatment. The antibody bound weakly to NCS peptides 1–2 and 1–3, but not peptides 1 or 2–4 (Fig. 1c, lane 7). This suggests that the epitope may include the NCS cleavage site between peptides 1 and 2. It is possible that 6H9 is actually a member of class B.

MAbs are often used for immune precipitations. We screened the 15 antibodies for their ability to precipitate p19. Medium from Rous sarcoma virus-infected chick embryo fibroblasts metabolically labelled with [35S]methionine was adjusted to 1% Triton X-100 to disrupt virus particles and then diluted in 10 mm-Tris-HCl pH 7.2 containing 1% NP40, 0.5% sodium deoxycholate, 0.1 M-NaCl, 1 mM-EDTA. Aliquots were incubated with ascites fluid containing the various MAbs and immune precipitates were collected using Pansorbin. The immune precipitates were washed with 10 mm-Tris–HCl pH 7.2 containing 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 0.15 M-NaCl, 1 mM-EDTA, and subjected to SDS–PAGE. Consistent with earlier results (Olsen, 1986) we found that all the University of Pennsylvania antibodies precipitated p19 under these conditions. In similar experiments with the immune precipitation of p130, the gag–fps protein of Fujinami sarcoma virus, we found that antibodies of class A as well as 2A7 failed to precipitate significant quantities of this protein from crude extracts of transformed rat cells. Perhaps access to the epitope is physically obscured by the additional amino acids present in this gag–one fusion protein.

Of the numerous approaches to the mapping of epitopes on a polypeptide (Eisenberg et al., 1985; Galvin et al., 1985; Jemmerson & Paterson, 1986; Parsons et al., 1986; Pierschbacher et al., 1981; Pinter et al., 1982; Reinfach & Fischman, 1985; Yurchenco et al., 1982), the method described here has the advantage of simplicity and rapidity. Successful application of the method requires that (i) the positions of methionine or tryptophan residues are known, (ii) the juxtaposition of methionine or tryptophan residues must be such that partial cleavage generates peptides that are representative of each of the complete cleavage products and that can be resolved by SDS–PAGE, and (iii) the MAbs of interest must produce adequate immunoblotting signals. For the collection of MAbs to ASLV p19 that we have examined, these requirements were largely satisfied. The classification of these antibodies with respect to approximate location of the epitope will be of use for further studies on the functional domains of p19 as well as studies on gag–one fusion proteins.

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REFERENCES


Short communication


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