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Cross-Reactivity of Antibodies Against Synthetic Peptides

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Antiserum against the synthetic peptide Lys-Arg-Ser-Arg-His-Phe, corresponding to the carboxy terminus of polyoma virus medium tumor antigen (medium T antigen), immunoprecipitates a protein of 36,000 daltons from polyoma virus-infected and uninfected cell extracts treated with the sulfhydryl group reagent N-ethyl-maleimide. This protein appears to share an antigenic determinant with medium T antigen that is normally buried inside the protein or covered up by another protein or cellular structure. The two-dimensional tryptic fingerprints of the 36K protein and of medium T antigen are apparently unrelated to each other. Antiserum against the octapeptide Ac-Met-Asp-Lys-Val-Leu-Asn-Arg-Tyr, including the amino-terminal heptapeptide sequence of the simian virus 40 (SV40) large tumor (T) and small T antigens, cross-reacts with polyoma virus large T antigen, which has an identical amino-terminal heptapeptide sequence except that Lys is replaced by Arg and Asn by Ser. The problem of cross-reactivities of antipeptide sera is discussed.

Key words: SV40, polyoma, tumor antigens, cellular proteins, immunoprecipitation

During the last four years we have explored the possibilities for producing site-specific antisera against proteins by immunizing with synthetic peptides corresponding to specific regions of the proteins [1]. The proteins chosen for these studies were three virus-coded transforming proteins: simian virus 40 (SV40) large tumor (T) antigen (SV40 large T antigen), polyoma virus medium tumor (T) antigen (medium T antigen) [2], and more recently, p60src of Rous sarcoma virus (Sefton and Nigg, unpublished data). The amino acid sequences of these proteins were deduced from the known nucleotide sequences of the DNA of the respective viral genomes [3–7]. In the case of SV40 large T antigen, two peptides corresponding to the amino- and carboxy-terminal ends of the polypeptide chain, eight and eleven amino acids long, respectively, were used as immunogens; in the other two cases antisera were produced against short peptides corresponding to the carboxy termini of the proteins. In all cases the antisera raised against the peptides also reacted with the respective native

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proteins. These antisera have proven to be useful tools in our study of the biological and structural properties of the transforming proteins. For instance we were able to purify polyoma virus medium T antigen by affinity chromatography 2,500-fold in one step using antipeptide serum in combination with peptide [8].

In the course of our studies on medium T antigen we observed that antibodies against the peptide Lys-Arg-Ser-Arg-His-Phe (anti-Lys-Phe), corresponding to the six carboxy-terminal amino acids of medium T antigen, recognized not only medium T antigen but also two cellular proteins of 30,000 and 26,000 daltons present in both polyoma virus infected and uninfected mouse cells [2]. In this communication we report that a third cellular protein of 36,000 daltons is immunoprecipitable with anti-Lys-Phe serum. We also demonstrate cross-reactivity of antiserum against a synthetic peptide, corresponding to the amino terminus of SV40 large T antigen, with polyoma virus large T antigen.

MATERIALS AND METHODS

Cells and Viruses

Mouse 3T6 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum. Polyoma virus stocks of the large plaque wild-type (WT) and the viable deletion mutant dl 8 were prepared from plaque isolates in cultures of 3T6 cells. The dl 8 mutation removes approximately 90 base pairs of DNA at about 90 map units of the polyoma virus genome and shortens the large and medium T antigens without affecting the small T antigen [9,10].

Preparation of Cell Extracts, Immunoprecipitation, and Tryptic Peptide Analysis

Infected and mock-infected 3T6 cells were radiolabeled for 3 hr starting 21 hr postinfection in DMEM lacking methionine, supplemented with 5% dialysed calf serum, and containing 250 μCi [^{35}S]-methionine per ml. Cell extracts were prepared in 10 mM Tris buffer (pH 7.5) containing 0.15 M NaCl, 1% DOC, 1% NP40, 0.1% SDS, and 1% Trasylol [11]. Treatment of extracts with N-ethyl-maleimide (NEM), a sulfhydryl group reagent, was carrried out by adding NEM to a final concentration of 10 mM and incubating for 1 hr at 4°C. After the incubation period cysteine was added to a final concentration of 12 mM. Immunoprecipitations and the analysis of the precipitates in SDS polyacrylamide slab gels were done as described [11]. The affinity purification of anti-Met-Tyr was carried out by Gabriele Grob as part of her Diplomarbeit at the University of Freiburg. The flow through material from the affinity chromatography column was used as control serum [2]. Preparative immunoprecipitations for isolating medium T antigen and 36K protein and the tryptic peptide analysis were performed as described [12].

Antisera

Antisera against the following two synthetic peptides were used: Lys-Arg-Ser-Arg-His-Phe (Lys-Phe), corresponding to the six carboxy-terminal amino acids of the medium T antigen of polyoma virus [2]; and Ac-Met-Asp-Lys-Val-Leu-Asn-Arg-Tyr (Met-Tyr) including the amino-terminal heptapeptide sequence of SV40 large T antigen [1]. The tyrosine residue was introduced to allow coupling of the peptide to

bovine serum albumin with bisdiazotized benzidine. The synthesis of the peptides, their coupling to bovine serum albumin for immunization, and the fractionation of the antisera by affinity chromatography have been published [1,2]. Antipolyoma tumor serum, a gift from Arthur Horwich and Dennis Templeton, was prepared in Brown Norwegian rats inoculated with polyoma-induced tumor cells.

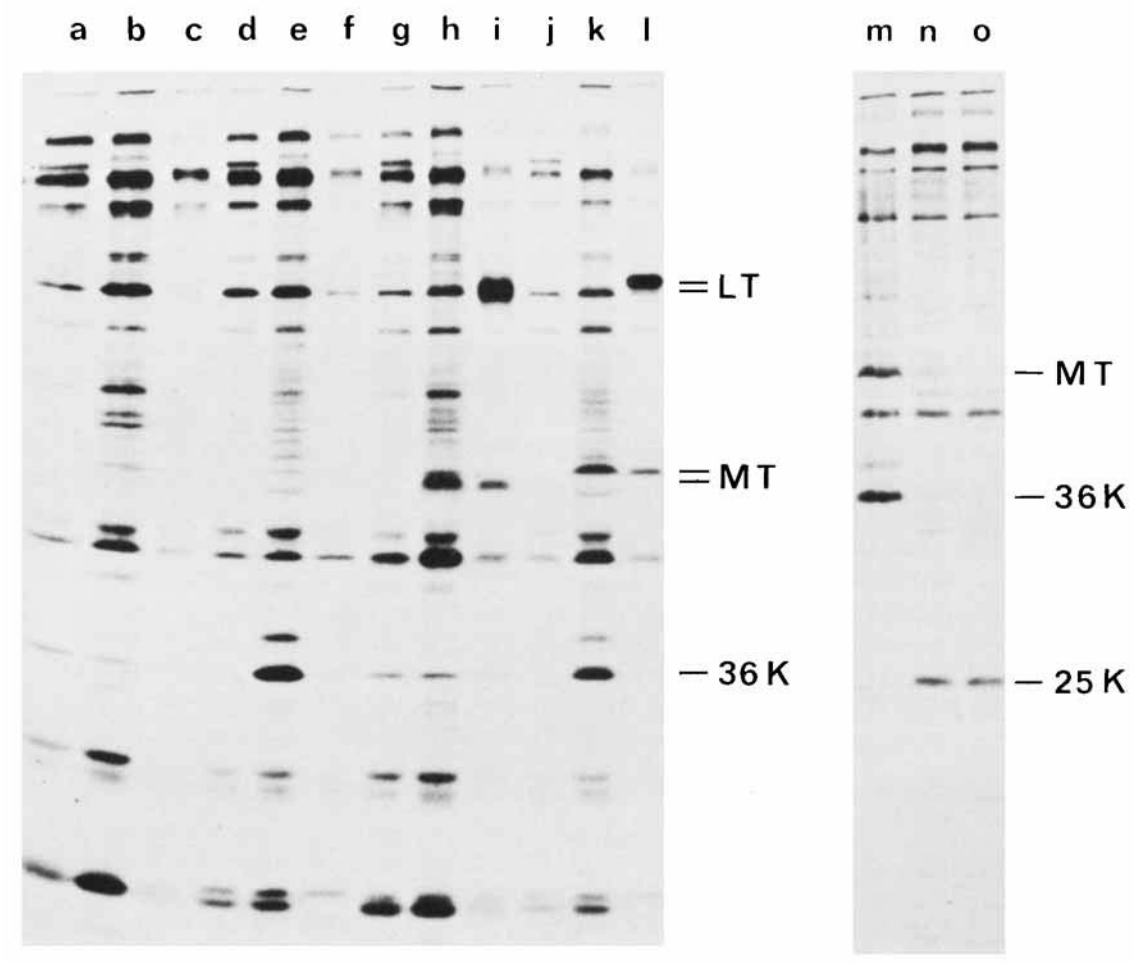

Fig. 1. Immunoprecipitation of the 36K protein from polyoma virus-infected and uninfected cell extracts. Lanes a, b, and c–mock-infected cell extracts; lanes d, e, and f–mock-infected, NEM-treated cell extracts; lanes g, h, and i–WT-infected cell extracts; lanes j, k, and l–WT-infected, NEM-treated cell extracts; lanes m, n, and o–dl 8-infected, NEM-treated cell extracts. Lanes a, d, g, and j represent immunoprecipitations carried out with control serum; lanes b, e, h, k and m with anti-Lys-Phe; lanes c, f, i, and l with antitumor serum; and lanes n and o with anti-Lys-Phe in the presence of 0.1 μg and 1 μg Lys-Phe-peptide, respectively. The lower portions of the gels are not shown. LT = large T antigen; MT = medium T antigen.

RESULTS

Cross-Reaction Between Medium T Antigen and a 36,000-Dalton Cellular Protein

We have previously observed that antiserum against the amino terminus of SV40 large T antigen precipitates large but not small T antigen although both proteins have identical amino acid sequences at their amino termini [1,13–15]. Presumably, the amino terminus of small T antigen is in a different conformational state than that of large T antigen or it may be sterically inaccessible to the antibodies due to interaction with other parts of the polypeptide chain or with other proteins. However, treatment of the SV40-infected cell extract with 10 mM N-ethyl-maleimide (NEM), a sulfhydryl group reagent, renders small T antigen precipitable (Walter, unpublished data) presumably by inducing a conformational change in small T antigen or by altering its protein–protein interactions. To investigate whether the antiserum against the carboxy terminus of medium T antigen (anti-Lys-Phe) also recognizes some "hidden" determinant on cellular proteins, mouse cell extracts were treated with NEM prior to immunoprecipitation. As shown in Figure 1, (lanes e, k, and m), under these conditions a prominent band of 36,000 daltons was present in precipitates from uninfected and polyoma virus infected cell extracts. Without NEM this protein did not precipitate (lanes b and h).

It should be pointed out that both polyoma virus large and medium T antigens migrate more slowly on polyacrylamide gels when precipitated from NEM-treated extracts. Large T antigen from untreated cell extracts migrates very close to an unrelated cellular protein. A protein of 25,000 daltons (lanes n, o) precipitates only in the presence of competing peptide as described previously [2]. It should also be mentioned that the previously described cross-reacting cellular proteins of 30,000 and 26,000 daltons [2] vary in amount depending on the extract and the particular serum. They are not detectable in Figure 1. In contrast, the 36K protein was always present in large quantity in all cell extracts and was precipitable with all sera from different rabbits immunized with Lys-Phe-peptide. Tryptic fingerprint analysis of [^{35}S]-methionine-labeled 36K protein showed no relationship with medium T antigen (Fig. 2). Preliminary studies indicate that this protein is not phosphorylated and that it is located in the nucleus. It was also detected in rat and hamster cell extracts.

Cross-Reaction Between SV40 and Polyoma Virus Large T Antigens

Antiseruum against Met-Tyr-peptide, corresponding to the amino terminus of SV40 large T antigen readily precipitated large T antigen as shown previously [1]. It also precipitated large T antigen of polyoma virus as shown in Figure 3. Precipitation was inhibited by Met-Tyr-peptide and is therefore specific. The anti-Met-Tyr serum was much less efficient in precipitating polyoma virus large T antigen than SV40 large T antigen. It was also less efficient than polyoma virus tumor serum in precipitating polyoma virus large T antigen.

DISCUSSION

The homology between medium T antigen and the 36K protein may be restricted to one short region only or may be more extensive and therefore of functional significance. The data presented do not allow us to distinguish between these two possibilities. The fact that the tryptic fingerprints of the two proteins are unrelated

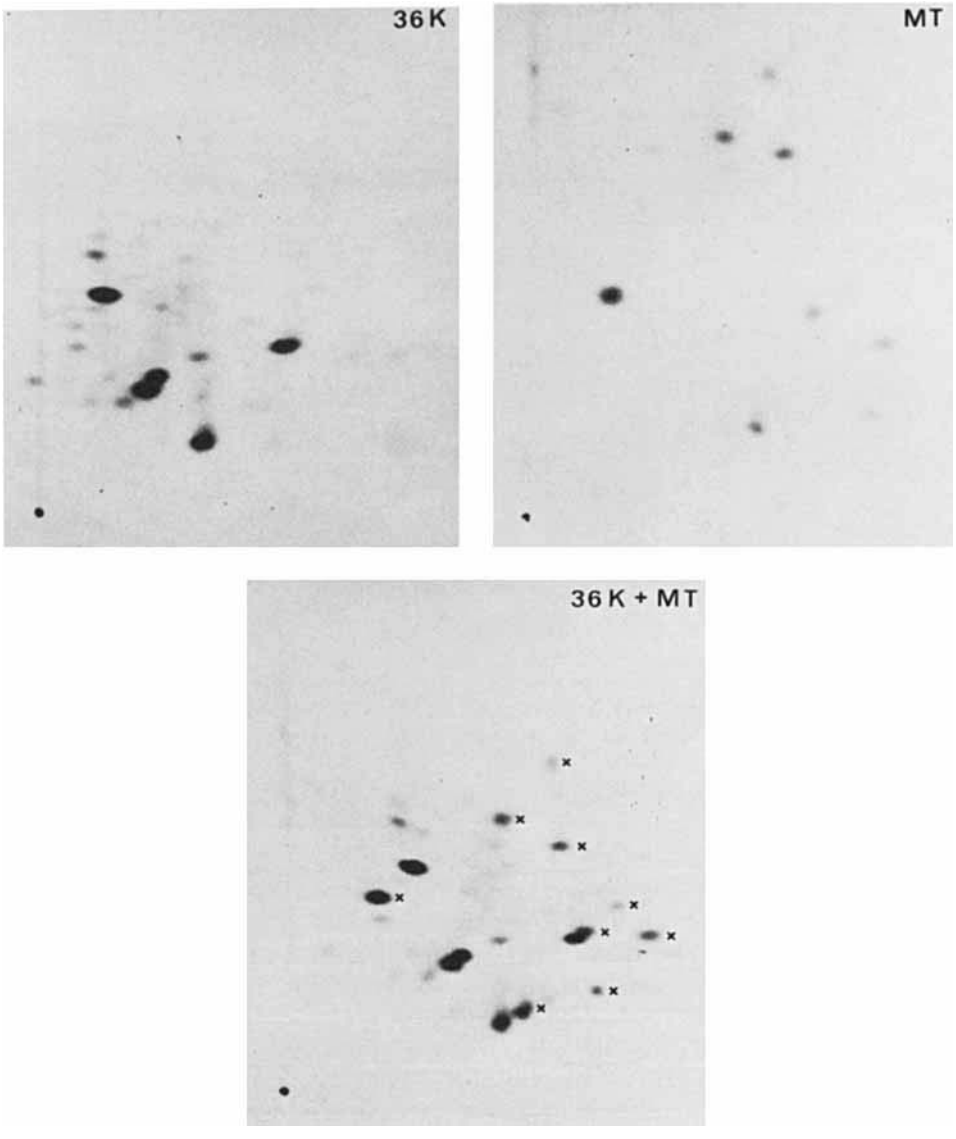

Fig. 2. Tryptic peptide analysis of the 36K protein and medium T antigen. Peptides from the medium T antigen are designated by "x" in the mixture of 36K protein and medium T antigen.

does not exclude extended homology. This was exemplified by comparison of the large and small T antigens of SV40 with those of polyoma virus that share no common methionine-containing tryptic peptides although they are clearly related by sequence. There is no simple solution to this question. If antibodies against other regions of medium T antigen could be raised that cross-react with the 36K protein, then the homology between the two proteins might not be considered fortuitous. Cross-

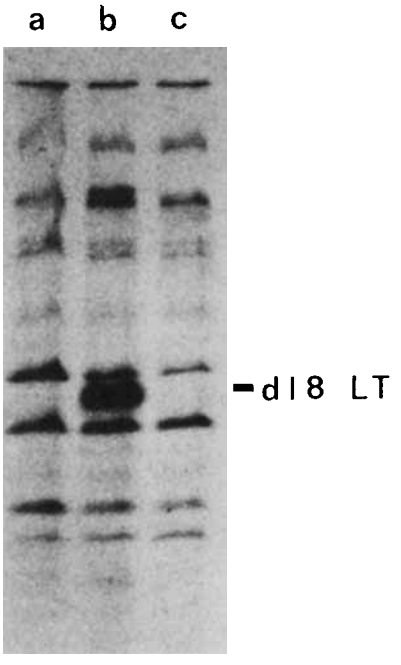

Fig. 3. Immunoprecipitation of polyoma virus large T antigen by anti-Met-Tyr. Precipitations were carried out with anti-Met-Tyr control serum (lane a), anti-Met-Tyr (lane b), and anti-Met-Tyr in the presence of 10 μg Met-Tyr-peptide (lane c). The lower portion of the gel is not shown. LT = large T antigen.

reactivity has also been observed with antibodies against a synthetic peptide corresponding to the carboxy terminus of p60src, the transforming protein of Rous sarcoma virus. These antibodies precipitated, in addition to p60src, two cellular phosphoproteins (Sefton, unpublished data) and, when used at higher concentration, three cytoskeletal proteins, myosin, tubulin, and vimentin (Nigg, unpublished data). The interaction of the antibodies with these proteins was inhibited by peptide. In the case of tubulin the observed cross-reactivity is likely to be fortuitous.

The finding that an antiserum against the amino terminus of SV40 large T antigen also precipitated polyoma virus large T antigen was not unexpected since the sequence of the amino-terminal eight amino acids of polyoma virus large T antigen is identical with that of SV40 large T antigen except that Lys and Asn at position 3 and 6, respectively, in SV40 large T antigen are replaced by Arg and Ser, respectively, in polyoma virus large T antigen. However, the degree of cross-reaction is low.

We do not understand all aspects of the observed cross-reactions. Most likely, immunization with an eight-amino-acid peptide elicits an immune response against a large number of determinants located on overlapping segments of the peptide, or consisting of different conformations of the peptide or segments thereof. Only particular subclasses of the antibody population might interact with protein, and these might recognize only a subclass of the protein. One might expect that antiserum against an eight-amino-acid peptide would cross-react with a large number of similar sequences

if this antiserum recognized all related sequences differing from each other in two amino acids at any of the eight positions. The number of possibilities for cross-reaction could be reduced either by introducing conformational restraints in addition to sequence specificity or by raising monoclonal antibodies against synthetic peptides and selecting those that are most restricted by sequence and/or conformation. We are presently trying the latter approach. Despite problems with cross-reaction, in our experience antibodies against synthetic peptides have always preferentially precipitated those proteins from which the peptide sequences were derived, and the number of proteins cross-reacting with high affinity appeared to be small. Nonspecific background could be distinguished in most cases from specifically precipitated proteins by inhibition of the specific precipitate, but not of the background, by the addition of peptide.

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