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Relationship Among Tau Antigens Isolated from Various Lines of Simian Virus 40-Transformed Cells

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In addition to the virus-specified tumor antigens, simian virus 40-transformed cells contain at least one other protein which can be immunoprecipitated with serum from animals bearing simian virus 40-induced tumors. This protein, which is designated Tau antigen, has an apparent molecular weight of 56,000 as determined by electrophoresis on acrylamide gels. The relationship among Tau antigens isolated from different lines of simian virus 40-transformed cells was examined by comparing the methionine-labeled tryptic peptides of these proteins by two-dimensional fingerprinting on thin-layer cellulose plates. In this fashion, we initially determined that the Tau antigens isolated from three different lines of transformed mouse cells were very similar. Second, we found that Tau antigen isolated from a line of rat transformants was closely related, but not identical, to the mouse cell Tau antigens. Approximately 70% of their methionine peptides comigrated in two dimensions. Finally, we showed that Tau antigen isolated from a line of transformed human cells was only partially related to the mouse and rat proteins. About 40% of the methionine peptides of the human protein were also contained in the Tau antigens from the other two species. These results strongly indicate that the Tau antigens isolated from these various simian virus 40-transformed cell lines contain common amino acid sequences.

Monkey cells infected with simian virus 40 (SV40) synthesize two proteins (94,000 and 20,000 daltons) which can be immunoprecipitated with serum from animals bearing SV40-induced tumors (anti-T serum) (1, 4, 29). These proteins are the large and small tumor antigens (T-Ag's) and are coded by the region of the SV40 genome that is expressed early in lytic infection and in virus-transformed cells (22, 23).

In addition to the T-Ag's, cells transformed by SV40 synthesize one other protein (50,000 to 56,000 daltons) which can be immunoprecipitated with anti-T serum (5, 9, 10, 16, 17, 19, 21, 28). This protein is probably coded by the cell DNA since it contains very few (zero to two) methionine-labeled tryptic peptides in common with either large or small T-Ag (5, 16, 28) and since uninfected mouse embryo carcinoma cells manufacture a protein that is very similar or identical to the 50,000- to 56,000-dalton protein isolated from SV40-transformed mouse cells (19). We have previously called this protein Tau antigen to distinguish it from the virus-coded T-Ag's (5). Smith et al. (28) have recently called this protein nonviral T-Ag.

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Tau antigens have been detected in all lines of SV40-transformed rat, human, hamster, and mouse cells that have been examined (5, 16, 21, 28), although transformed hamster cells appear to make smaller quantities of these proteins (28). In addition, cells transformed by other viruses (Moloney murine leukemia or sarcoma virus) or by chemicals (methylcholanthrene) synthesize similarly sized proteins which may be Tau antigens (7). These proteins are apparently not produced in spontaneously transformed mouse cells (5), in SV40-transformed cells which have reverted to a T-Ag-negative state (5), or in normal untransformed cells (7, 19, 28). A portion of the Tau antigen in transformed cells may be found in a complex with large T-Ag (17).

We investigated the relationship among Tau antigens isolated from various lines of SV40-transformed cells to determine whether these proteins are identical. For this purpose, we examined the methionine-labeled tryptic peptides of Tau antigens isolated from three different lines of SV40-transformed mouse cells, one line of transformed rat cells, and one line of transformed human cells. We found that these proteins were not all identical, and yet they all contained some peptides in common. In addition, we noticed that the mouse-derived Tau

antigens were nearly identical to one another, that the rat protein was significantly related to, but clearly distinguishable from, the mouse proteins, and that the human protein was only partially related to the others.

MATERIALS AND METHODS

Cells. SV40-transformed BALB/c and AL/N mouse cells (lines 315 and 215, respectively) have been described previously (5). BALB/c (line 11A8) transformants were obtained from G. Todaro. Human transformed cells (line SV80) were isolated originally by Todaro et al. (30), and rat transformed cells were isolated by W. Topp and described by Botchan et al. (3).

Immunoprecipitation and gel electrophoresis. Transformed cells were grown in 150-cm^2 culture flasks and labeled for 3 h with 50 μCi of L-[^{35}S]methionine per ml, as previously described (5). The cells in each flask were washed three times with ice-cold 0.01 M Tris–0.001 M Na_2HPO_4–0.137 M NaCl, pH 7.4 (Tris-buffered saline), and lysed during a 15-min period at 0°C in the presence of 2 ml of extraction buffer (5). The lysate was collected and centrifuged at 150,000 × *g* for 40 min at 2°C. The supernatant was carefully removed and incubated at 0°C for 1 h with 25 μl of either normal or anti-T hamster serum. The anti-T serum was obtained from hamsters carrying SV40-induced tumors and was provided by J. Gruber, National Cancer Institute.

Protein A-bearing *Staphylococcus aureus* (Cowan I strain; NCTC 8530) was prepared by the method of Jonsson and Kronvall (14). The bacteria were washed three times at 0°C with extraction buffer, resuspended in the same buffer to a final concentration of 10% (wt/vol), and added (300 μl) to the reaction mixture to precipitate the immune complexes. After 1 h at 0°C, the bacteria were washed once in extraction buffer, six times in 0.1 M Tris–0.5 M LiCl–1% 2-mercaptoethanol, pH 9.0 (26), and once in Tris-buffered saline (pH 7.4) and suspended in 150 μl of 0.075 M Tris-sulfate (pH 8.6)–2% sodium dodecyl sulfate–2% 2-mercaptoethanol–0.002% bromophenol blue–15% glycerol (20).

Gel electrophoresis and chromatography of tryptic peptides. Protein samples were heated and subjected to electrophoresis for 2.5 to 4 h at 25 mA in gels containing 13% acrylamide and 0.26% bisacrylamide as described by Tegtmeyer et al. (29). Proteins to be characterized further were extracted from the gel and digested with trypsin as previously described (27). Tryptic peptides were separated in two dimensions on thin-layer cellulose plates essentially as described by Gibson (11). The peptides in water-pyridine-acetic acid (300:10:3, vol/vol), pH 5.4 (12), were applied near the corner of a thin-layer plate and subjected to electrophoresis at 4°C in the same buffer for 3 h at 300 V. Chromatography in the second dimension was performed in butanol-pyridine-water-acetic acid (97:75:60:15, vol/vol) pH 5.3 (11), for 5.5 to 6 h at room temperature. After thorough drying in a fume hood, the plates were dipped in molten (40°C) 2-methylnaphthalene containing 0.4% 2,5-diphenyloxazole, as described by Bonner and Stedman (2). Kodak XR2 film was exposed to the plates for 1 to 3 weeks at −70°C before being developed.

RESULTS

Immunoprecipitation of Tau antigens. Tau antigens are cellular proteins that are synthesized in a variety of SV40-transformed (5, 16, 17, 19, 28) and non-SV40-transformed (7, 19) cells. However, they have not been detected in normal, untransformed cells (7, 19, 28). Since the Tau antigens isolated from various transformed cells have the same approximate molecular weight (50,000 to 56,000), we were interested in determining whether the same protein was made in all cells transformed by SV40 or whether the proteins varied among different cell lines. Furthermore, if differences were found, we wanted to determine the relationship among Tau antigens isolated from various lines of cells of the same species and of different species. We therefore examined the peptides of the Tau antigens isolated from three lines of mouse transformants (lines 11A8, 315, and 215; derived from BALB/c, BALB/c, and AL/N mouse strains, respectively), one line of transformed rat cells (line 14B), and one line of transformed human cells (line SV80). These proteins were immunoprecipitated from extracts of cells labeled with L-[^{35}S]methionine and subjected to electrophoresis (Fig. 1) in acrylamide gels. Figure 1 shows that each of these cell lines contained one or more forms of Tau antigen (54,000 to 56,000 daltons) that were immunoprecipitated with anti-T serum but not with normal serum. In particular, lines 215, 315, and SV80 contained at least two forms of immunoprecipitable Tau antigens (Fig. 1b through d). All cell lines also contained a large T-Ag with an apparent molecular weight of 94,000. Larger forms of T-Ag were also apparent in lines 11A8 (100,000 daltons) and 315 (130,000 daltons) (Fig. 1a and c). These and other high-molecular-weight forms of large T-Ag have been detected previously in various lines of SV40-transformed cells (5, 15, 16, 18, 24, 28). The small T-Ag of SV40 was not readily detected in these immunoprecipitates, partially because the anti-T serum used was chosen for maximum precipitation of Tau antigens and partially because the amounts of anti-T serum used were too small to precipitate small T-Ag quantitatively (6).

Two-dimensional fingerprinting of methionine-labeled tryptic peptides. To compare the peptides of Tau antigens isolated from various lines of SV40-transformed cells, labeled immunoprecipitated proteins were applied to preparative acrylamide gels. The bands corresponding to the slower-migrating Tau antigen

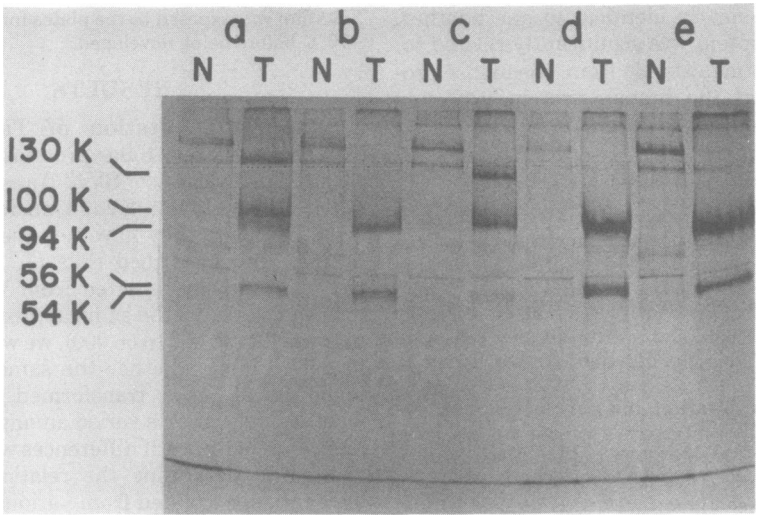

FIG. 1. *Acrylamide gel electrophoresis of proteins immunoprecipitated from various lines of SV40-transformed cells: labeled proteins precipitated with either normal (N) or anti-T (T) hamster serum from SV40-transformed line 11A8 (a), 215 (b), 315 (c), SV80 (d), and 14B (e) cells. SV40-transformed mouse cells (lines 11A8, 315, and 215), rat cells (line 14B), and human cells (line SV80) were labeled for 3 h with L-[^{35}S]-methionine. Extracts were prepared and incubated with either normal or anti-T hamster serum, followed by incubation with protein A-bearing S. aureus. Proteins in the washed precipitates were subjected to acrylamide gel electrophoresis for 2.5 h at 25 mA, and the labeled proteins in the gel were detected by exposure to X-ray film. Molecular weights were estimated by the relative rates of migration of marker proteins with known molecular weights (5). 130K = 130,000 daltons.*

species of each cell line (Fig. 1) were excised from the gels, and the protein was extracted and treated with trypsin. The resulting peptides were separated in two dimensions by electrophoresis and chromatography (fingerprinting) on thin-layer cellulose plates (11). Methionine-labeled peptides were detected on the plates by fluorography, using 2-methylnaphthalene (2). To compare any two proteins, peptide samples were analyzed separately on different plates and as a mixture on a third plate. Figure 2 shows the fingerprints of the methionine-containing tryptic peptides of the Tau antigens isolated from the mouse transformants 11A8 (Fig. 2A), 315 (Fig. 2B), and 215 (Fig. 2C). The fingerprint of a mixture of the Tau antigen peptides derived from cell lines 11A8 and 215 is shown in Fig. 2D. The methionine-labeled tryptic peptides of the Tau antigens from two of these cell lines (lines 11A8 and 215) were indistinguishable (Fig. 2A, C, and D). Those derived from the third mouse cell line (line 315) (Fig. 2B) were slightly different in that one peptide (peptide a, Fig. 2A and C) was absent. The appropriate mixing experiment showed that the remaining peptides of that protein corresponded to the peptides derived from lines 11A8 and 215 (data not shown). Since lines 11A8 and 315 originated from the BALB/c strain of mice, whereas line 215 originated from the AL/N strain, the observed differences cannot be due simply to a difference in strains. Rather, they suggest that mouse Tau antigens might vary slightly from one another, irrespective of the strains from which the transformed cell lines were derived. The similarities of these fingerprints do indicate that the Tau antigens isolated from mouse transformants are very closely related proteins. This is in agreement with the data of Linzer and Levine (19), Smith et al. (28), and Chang et al. (5). The relationship among the peptides of the three mouse Tau antigens is shown below (see Fig. 5a).

In a similar manner, the Tau antigens isolated from the rat and human transformants were compared with each other and with those of the mouse cell lines. Figures 3A through C show that the rat (line 14B) Tau antigen contained 16 methionine peptides (Fig. 3B) that were also present in mouse (line 11A8) Tau antigen (Fig. 3A) and 7 peptides that were different. The Tau antigen from human line SV80, on the other hand, contained only eight methionine peptides (Fig. 3D) that were also present in the mouse protein (peptides 1, 3, 4, 6, 12, 13, 14, and 16) (Fig. 3A, D, and E). These eight peptides were also present in the rat Tau antigen (Fig. 3B, D,

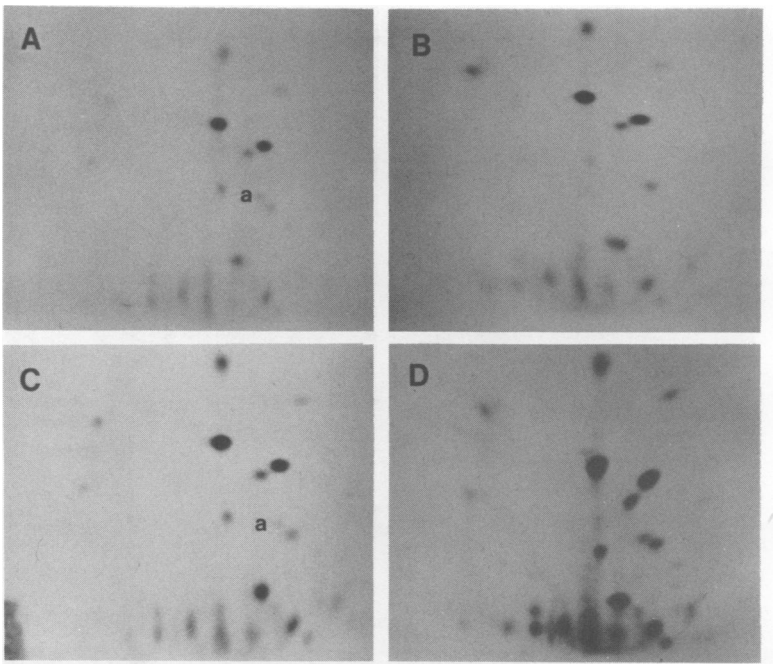

FIG. 2. *Fingerprints of methionine-labeled tryptic peptides of mouse Tau antigens isolated from line 11A8 (A), 315 (B), 215 (C), and 11A8 plus 215 (D) cells. SV40-transformed mouse cell lines 11A8, 315, and 215 were labeled with L-[^{35}S]methionine, and the extracts of these cells were incubated with hamster anti-T serum in separate immunoprecipitation reactions. Tau antigens from each of these cell lines were isolated from preparative acrylamide gels and treated with trypsin, and the resulting peptides were applied near the corner of a thin-layer cellulose plate (11). Electrophoresis was carried out at 300 V for 3 h at 4°C. The direction of electrophoresis was from left to right near the bottom of each fingerprint. This was followed by ascending thin-layer chromatography (from bottom to top in the fingerprints). Plates were dipped in 2-methylnaphthalene and exposed to X-ray film at −70°C for about 3 weeks.*

and F) and were therefore a subset of the 16 peptides common to the mouse and rat Tau antigens. These data are summarized below (see Fig. 5b).

The relationship between the different electrophoretic forms (56,000 and 54,000 daltons) of the Tau antigens isolated from transformed human cells (Fig. 1d) was investigated by comparing their methionine-labeled tryptic peptides. For preparative purposes these proteins were separated from one another by subjecting the samples to electrophoresis for a longer period of time than for the gel shown in Fig. 1. Figures 4A and B show the fingerprints of the methionine-containing tryptic peptides of the slower (56,000 daltons) and faster (54,000 daltons) species of line SV80 Tau antigen, respectively. The results of the mixing experiment are shown in Fig. 4C. These two proteins contained the same 18 peptides. The 56,000-dalton form of the protein contained an additional peptide (Fig. 4A and C, peptide b). This result is summarized below (see Fig. 5c).

DISCUSSION

An analysis of methionine-labeled tryptic peptides showed that the Tau antigens isolated from three different lines of transformed mouse cells were very similar. This is in agreement with previous observations made by us (5), by Linzer and Levine (19), and most recently by Smith et al. (28). We noticed in this study, however, as did Smith et al. (28), that all mouse Tau antigens were not identical. The Tau antigen isolated from line 315 lacked one of the peptides (Fig. 1, peptide a) present in the proteins from the other two mouse lines (lines 11A8 and 215).

By comparing the peptides of mouse cell Tau antigen (line 11A8) with those of rat (line 14B) and human (line SV80) transformed cells, we found that the three proteins apparently contained the same eight methionine-labeled tryptic peptides (Fig. 5b). Furthermore, the mouse and rat Tau antigens appeared to be more closely related, sharing an additional eight peptides. The most straightforward interpretation of these

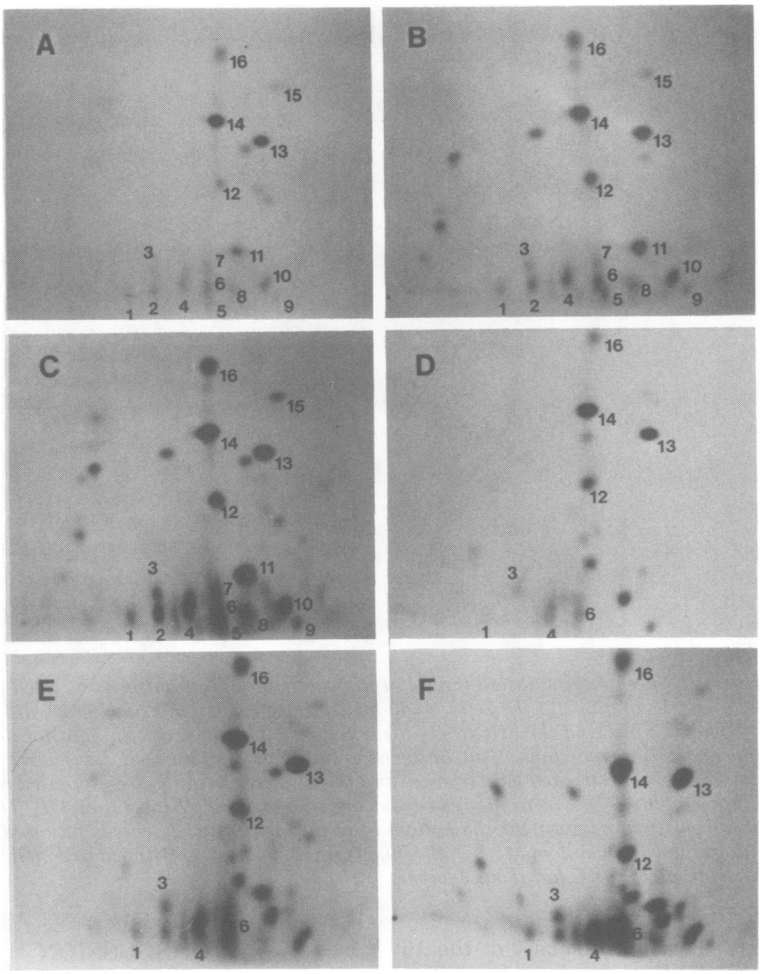

FIG. 3. *Comparison of methionine-labeled tryptic peptides of Tau antigens isolated from mouse, rat, and human transformants: fingerprints of methionine-labeled peptides of Tau antigens isolated from lines 11A8 (A), 14B (B), 11A8 plus 14B (C), SV80 (D), SV80 plus 11A8 (E), and SV80 plus 14B (F). Tau antigens were isolated from methionine-labeled transformed mouse (line 11A8), rat (line 14B), and human (line SV80) cells by immunoprecipitation and gel electrophoresis. Tryptic peptides were prepared and separated in two dimensions on thin-layer cellulose plates. The numbered peptides in (A), (B), and (C) refer to peptides common to mouse and rat Tau antigens. Those in (D), (E), and (F) refer to peptides present in mouse, rat, and human Tau antigens.*

results is that these Tau antigens are descendants of a single protein which has evolved differently in mice, rats, and humans. This situation would be analogous to the evolution of cytochrome *c* or hemoglobin (8), in which proteins from closely related species are more similar than those from distantly related species. This would explain the observed similarity in the Tau antigens of mouse and rat transformants and their more distant relationship to the human protein. The eight peptides that were found in all of the Tau antigens that we examined might correspond to regions in the proteins that are conserved to maintain proper structure and function.

Some lines of SV40-transformed cells contained more than one electrophoretic form of Tau antigen (Fig. 1). This observation has been made previously by us (5) and others (9, 16, 21, 28). Fingerprint analysis of the methionine-labeled tryptic peptides of the 56,000- and 54,000-dalton forms of the Tau antigens isolated from SV40-transformed human cells showed that these proteins were closely related (Fig. 4 and 5c). Therefore there may be a precursor-product relationship between these two forms of Tau

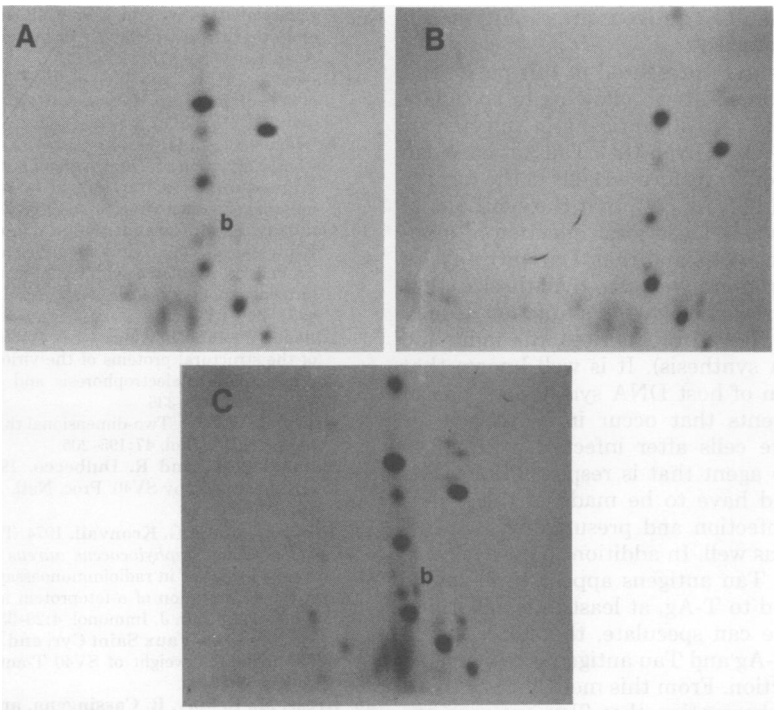

FIG. 4. *Comparison of the methionine-labeled tryptic peptides of two different forms of human Tau antigen: fingerprints of the methionine-labeled peptides of the 56,000-dalton (A), 54,000-dalton (B), and a mixture of the 56,000- and 54,000-dalton (C) Tau antigens isolated from line SV80 cells. Human transformed cells (line SV80) were labeled with L-[^{35}S]methionine, and extracts of these cells were incubated with anti-T serum in an immunoprecipitation reaction. The 56,000- and 54,000-dalton forms of line SV80 Tau antigen (Fig. 1) were separated by gel electrophoresis for 4 h at 25 mA. The tryptic peptides of these proteins were subjected to electrophoresis and chromatography on thin-layer cellulose plates.*

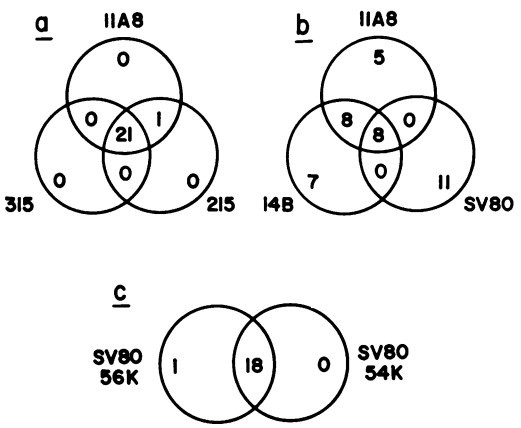

FIG. 5. *Relationships among the methionine-labeled tryptic peptides of mouse Tau antigens (a), mouse, rat, and human Tau antigens (b), and human 56,000-dalton (56K) and 54,000-dalton (54K) Tau antigens (c). The numbers in the areas where three circles overlap in (a) and (b) refer to the numbers of peptides common to the three species of Tau antigen indicated. The numbers of peptides common to only two of the three proteins are indicated in the other regions where the circles overlap. The numbers of peptides unique to individual proteins are indicated outside the overlapping regions. In (c), in which only two proteins are compared, the number within the region of overlap refers to the number of peptides common to these two proteins.*

antigen, or the proteins may be products of nearly identical genes. Pulse-chase experiments or labeling experiments performed in the presence of protease inhibitors might distinguish between these possibilities.

Recent data from our laboratory (manuscript in preparation) indicate that a Tau antigen protein can be immunoprecipitated from monkey cells after infection with SV40. These results imply that Tau antigens are induced in either permissive or nonpermissive (i.e., mouse [19]) cells after infection with SV40. An analysis of the methionine-labeled tryptic peptides of the Tau antigen isolated from infected monkey cells showed that this protein is very closely related to the Tau antigens from transformed human cells (line SV80). This observation supports our

contention that Tau antigens are evolutionarily conserved proteins.

The information presented in this paper and the evidence cited above allow us to speculate about the role of Tau antigens in infected or transformed cells. Given that Tau antigens can be isolated from transformed cells in the form of a complex with T-Ag (17), that the synthesis of Tau antigens is induced after infection of monkey cells with SV40, and that Tau antigens are conserved proteins, we can hypothesize that these proteins are involved in a function usually attributed to T-Ag (for example, the induction of host DNA synthesis). It is well known that the activation of host DNA synthesis is one of the early events that occur in permissive or nonpermissive cells after infection with SV40 (13, 25). The agent that is responsible for this process would have to be made in these cells early after infection and presumably in transformed cells as well. In addition to meeting this requirement, Tau antigens appear to have the ability to bind to T-Ag, at least in transformed cells (17). We can speculate, therefore, that a complex of T-Ag and Tau antigen is responsible for this induction. From this model, it is easy to explain the observation that Tau antigens are conserved proteins since they would have to interact with SV40 T-Ag, as well as with the DNA-synthesizing machinery of many different types of cells.

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