in the activation of different effector functions.

We next examined the ability of cultured and of freshly isolated monocytes to kill human tumor cells in the presence of tumor-specific monoclonal antibodies derived from mice. Cultured monocytes and macrophages from human donors M.K. and C.R. exhibited cytolytic activity in the presence of an IgG2a monoclonal antibody against colorectal carcinoma cells (Table 3). In addition, monocytes (24-hour) and macrophages freshly isolated from ascites of a patient (J.R.) with metastatic colon carcinoma reproducibly exhibited a low level of cytolytic activity in the presence of tumor-specific IgG2a antibody (Table 3). Cultured monocytes and macrophages and antibodies of the IgG2b and IgG1 subclasses mediated some destruction of tumor cells, although at much lower levels compared to IgG2a antibody. However, an IgG2a antibody that does not bind to colorectal cancer cells did not mediate tumor cell destruction.

We have thus demonstrated that human macrophages can mediate the killing of human tumor cells in vitro in the presence of tumor-specific, monoclonal IgG2a antibodies derived from mice. Our results strongly support the hypothesis (*14*) that murine IgG2a monoclonal antibodies directed against human tumor antigens may be effective in the immunotherapy of human cancer.

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Genetic Mapping of the Mouse Proto-Oncogene c-*sis* to Chromosome 15

Abstract. *The mouse homolog (*c-sis*) of the transforming gene of the simian sarcoma virus was mapped to chromosome 15 by the Southern blot analysis of DNA's from hamster-mouse somatic cell hybrids. Alterations in* c-sis *expression may thus play a role in the various murine neoplastic diseases characterized by rearrangements or duplications of chromosome 15.*

The genomes of the rapidly transforming (acute) retroviruses contain transforming genes (v-*onc* genes) that are derived from a set of chromosomal sequences (c-*onc* genes). Molecular analysis of these viruses has led to the identification and characterization of more than 15 different v-*onc* genes which can induce various types of tumors in vivo and transform cells in vitro (*1*). The cellular homologs of these sequences are highly conserved among vertebrate species; they are thought to function in normal cellular and developmental processes; and their coding regions, like those of other cellular genes, are separated by intervening sequences (*1*).

Table 1. Correlation between specific mouse chromosomes and the c-*sis* mouse homolog in 19 somatic cell hybrids.

Mouse chromosomes	Number of hybrid clones: Mouse c-*sis*/chromosome retention +/+	−/−	+/−	−/+	Percent discordant
1	10*	2	7	0	37
2	8	2	9	0	47
3	8	2	9	0	47
4	6	2	11	0	58
5	3	2	14	0	74
6	8	2	9	0	47
7	13	2	4	0	21
8	8	1	9	1	53
9	4	2	13	0	68
10	4	2	13	0	68
11	0	2	17	0	89
12	12	1	5	1	32
13	6	2	11	0	58
14	4	1	13	1	74
15	16	2	1	0	5
16	8	2	9	0	47
17	10	2	7	0	37
18	7	1	10	1	58
19	6	2	11	0	58
X	9	1	8	1	47

*Ten hybrids contain c-*sis* and chromosome 1 (+/+), two hybrids lack c-*sis* and chromosome 1 (−/−), and seven hybrids contain c-*sis* but not chromosome 1.

Although viral *onc* sequences are directly responsible for tumorigenesis by acute retroviruses, several lines of evidence suggest that neoplastic transformation can also result from the abnormal expression of cellular *onc* genes. First, c-*onc* genes such as c-*myc* can be activated by the local insertion of a nonacute retrovirus (*2*). Second, DNA's from certain human and animal tumors contain transforming genes which, in some cases, show homology with known v-*onc* genes (*3*). Third, expression of several cellular oncogenes is increased as a result of the amplification of oncogene sequences in tumor lines (*4*). Fourth, chromosomal rearrangements may relocate c-*onc* genes in transcriptionally active regions; different *onc* genes have now been chromosomally mapped at or near the breakpoints involved in these tumor-specific aberrations (*5*).

In the mouse, a number of neoplastic diseases are marked by karyotypic abnormalities involving chromosome 15, and attention has focused on the one oncogene known to be present on this chromosome, c-*myc* (*6*). We now report that the mouse homolog of another transforming gene, c-*sis*, is also present on chromosome 15.

The v-*sis* oncogene was originally found in the simian sarcoma virus isolated from a fibrosarcoma of a woolly monkey (*7*). To determine the chromosomal location of the cellular homolog of this oncogene in the mouse, we analyzed DNA's from hamster-mouse somatic cell hybrids by the Southern blot hybridization procedure (*8*). The cell hybrids were derived from the fusion of E36 Chinese hamster cells with cells of three different

mouse strains (*9*). The mouse chromosome content of each line was determined by Giemsa-trypsin banding followed by staining with Hoechst 33258 (*10*) and by the expression of isozyme loci on 13 mouse chromosomes (*9*).

A molecularly cloned 1.7-kilobase (kb) Bam HI fragment representing the 3′ exon of the human c-*sis* locus was used as a probe (*11*) (Fig. 1). Analysis of 19 independent hybrids demonstrates a strong correlation between chromosome 15 and the 6.9-kb Bam HI fragment containing the mouse c-*sis* homolog (Table 1). Most of the hybrids contained mouse c-*sis* (17 of 19 tested), consistent with the observation that chromosome 15 is preferentially retained in hamster-mouse hybrids (*12*). Only one discrepancy with chromosome 15 was observed. This hybrid contained mouse c-*sis* sequences, but karyotypic analysis failed to identify mouse chromosome 15. However, this hybrid must contain a fragment of mouse chromosome 15 because it contained mouse c-*myc* sequences as shown by blot hybridization (*13*). The only intact mouse chromosome present in this hybrid is chromosome 17, but data from the other hybrids clearly show no correlation between this chromosome and c-*sis*. Thus, the data show that c-*sis* is on chromosome 15.

Comparisons of the human and mouse linkage maps have identified regions of chromosomal homology between the two species. It was therefore initially unexpected that the oncogenes c-*myc* and c-*sis*, which are found on human chromosomes 8 (*14*) and 22 (*15*), respectively, both map to mouse chromosome 15. However, data from other sources indicate that human 22 and mouse 15 share regions of homology, since both chromosomes carry the genes for the enzymes NADH diaphorase and arylsulfatase A (*16*). These two loci are present at the distal end of the long arm of human chromosome 22 (22q13), and the human c-*sis* locus is also on the long arm of this chromosome (*11*). Our data are consistent with the interpretation that these linkage relationships are maintained on mouse chromosome 15.

Several neoplastic diseases of man are characterized by altered expression of c-*sis* or rearrangements of chromosome 22. Thus sarcomas and glioblastomas show an increase in c-*sis* messenger RNA, as does the T-cell lymphoma line HUT 102 (*17*). Other tumors such as some Burkitt's lymphomas and various hematopoietic neoplasms contain translocations involving the long arm of chromosome 22 (*18*); this same subchromosomal region carries the c-*sis* gene (*11*). However, altered expression of c-*sis* has not been detected in these tumors, and therefore the role of c-*sis* in the establishment and maintenance of transformation in these tumors is not clear.

In the mouse, abnormalities involving chromosome 15 are found in various neoplastic diseases. In T-cell leukemias, a 15;X translocation is found in lymphomas of the SJL mouse (*19*), and trisomy 15 is routinely found in the spontaneous thymic lymphomas of AKR mice and in the lymphomas induced in other inbred strains by x-rays, radiation leukemia viruses, or carcinogens (*20*). Among the B-cell diseases, reciprocal 12;15 translocations or, less frequently, 6;15 translocations have been identified in plasmacytomas (*21*). Rearrangements of the *myc* gene on chromosome 15 or its altered expression have been reported in most plasmacytomas and in some B-cell lymphomas (*22*). However, activation of another oncogene may also occur in these cells since c-*myc* is not the gene in plasmacytoma DNA that transforms NIH 3T3 cells (*6, 23*). Furthermore, c-*myc* is rarely rearranged in T-cell leukemias (*22*). Our finding that the c-*sis* gene is also on chromosome 15 raises the possibility that chromosome 15 abnormalities may alter expression of this oncogene. After this report was first submitted, *sis* was shown to be closely related to the gene coding for platelet-derived growth factor (*24*). These findings should prompt further studies to define the role of c-*sis* in murine neoplasms.

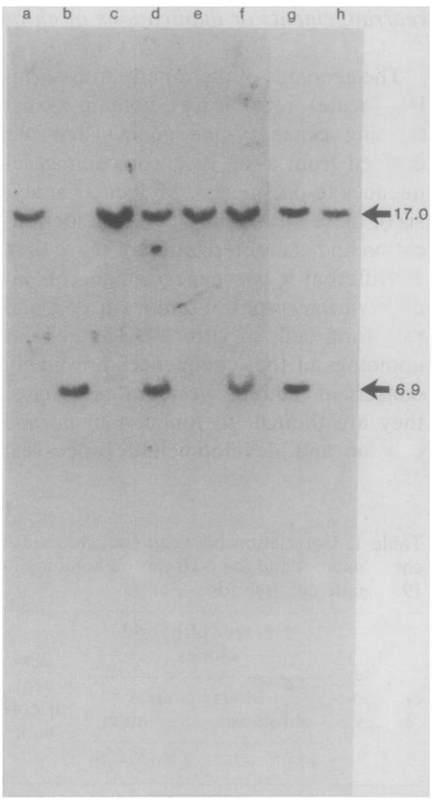

Fig. 1. Hybridization of cloned c-*sis* with Chinese hamster, mouse, and hybrid cell DNA's. High molecular weight DNA was prepared as described (*25*). DNA's were digested with Bam HI, subjected to electrophoresis through 0.4 percent horizontal agarose slab gels, transferred to nitrocellulose membranes, and hybridized to a ^{32}P-labeled human pc-*sis* DNA probe under the following conditions: 50 percent formamide, 10 percent dextran sulfate, and fivefold strength standard saline citrate with salmon sperm DNA (10 ng/ml) at 42°C for 18 to 24 hours. Membranes were washed two times with double-strength standard saline citrate and 1 percent sodium dodecyl sulfate at room temperature, then washed two to four times at 15°C for 15 minutes each time. (Lane a) Chinese hamster cell line E36; (lane b) BALB/c; (lane c) BALB hybrid BM14; (lane d) BALB hybrid BM34; (lane e) BALB hybrid BE7-2.3-11; (lane f) NFS hybrid VE8-1; (lane g) NFS hybrid VE21K; and (lane h) NFS hybrid VE12A.

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Angiotensin Synthesis in the Brain and Increased Turnover in Hypertensive Rats

Abstract. *The missing link in the evidence for an active endogenous renin angiotensin system in the brain has been the demonstration of local angiotensin synthesis in the central nervous system in vivo. In this report the extraction and characterization of angiotensin I and angiotensin II from the brain of rats is described. The accumulation of angiotensin I was enhanced in hypertensive rats when the conversion to angiotensin II was blocked in vivo by the converting enzyme inhibitor captopril.*

The renin angiotensin system (RAS) is still usually considered to be a circulating blood hormone system. Angiotensin II (ANG II) in the brain stimulates pituitary hormone release and sympathetic tone and thereby influences blood pressure and volume homeostasis (*1, 2*). These effects suggested that ANG II, like other peptide hormones, might also occur endogenously in the brain as a neuropeptide (*3*). Immunohistochemical evidence supported such a possibility (*4–8*), but biochemical data were controversial (*9, 10*) or negative (*11*).

We report here the presence of [Ile^5]ANG I, [Ile^5]ANG II, and [Ile^5]-ANG III in the brain of intact and bilaterally nephrectomized rats. We also studied the turnover of angiotensin in the brain and found that inhibition of converting enzyme (CE) led to an accumulation of ANG I in nephrectomized rats, thus proving that active endogenous synthesis of the peptide occurs in the brain. This effect was more marked in hypertensive than nonhypertensive rats, indicating that the brain RAS is stimulated in spontaneously hypertensive rats of the stroke prone strain (SHRSP). Our data thus show that ANG II is a neuropeptide that is locally synthesized in the brain.

Cerebrospinal fluid (CSF) was collected by puncture of the fourth brain ventricle (*12*) from one group of ten untreated rats and another group of rats that received, 15 minutes before the CSF sampling, an injection of 100 mU of hog kidney renin in the fourth ventricle. The generation of ANG I was measured in vitro in pooled CSF, that was incubated at 37°C for 4 hours with purified hog kidney renin and tris-maleate buffer, *p*H 5.5, containing angiotensinase inhibitors (*12*). ANG I and ANG II were measured in all samples by radioimmunoassay and the peptides were characterized by high-pressure liquid chromatography (HPLC).

Angiotensin was extracted from the brain of adult rats, each weighing 250 g. The animals were bilaterally nephrectomized and brain tissue was obtained 24 hours later. The brains were freed of blood by transcardiac perfusion with warm 0.9 percent saline containing 25 U of heparin per milliliter at 37°C and then quickly removed, dissected, and frozen on dry ice. For the extraction of angiotensin, tissue was homogenized in 0.1*N* HCl at a ratio of 1:10, weight to volume, boiled for 5 minutes, and centrifuged at 30,000*g* and 4°C for 30 minutes. The clear supernatant was collected and the pellet was reextracted with 0.1*N* HCl. The supernatants were pooled and purified on octadecasilyl-silica (ODS-silica) cartridges (Sep-Pak C 18, Waters Associates). In this procedure, the cartridges were carefully washed with 3 ml of methanol and 10 ml of a 1 percent trifluoracetic acid (TFA) solution in water. To minimize nonspecific adsorption, the cartridges were then coated with 1 ml of a 1 percent polypeptide solution (Serva, Heidelberg) and washed again with a

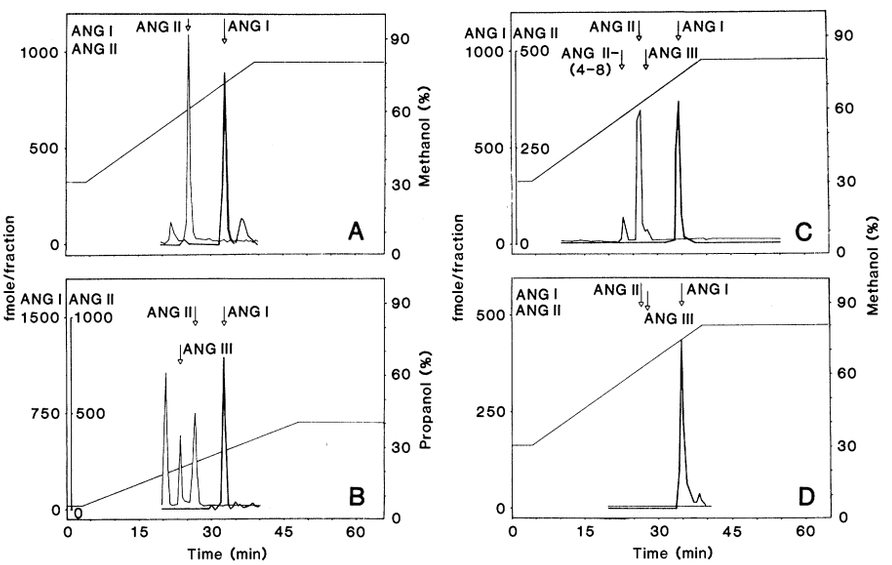

Fig. 1. (A and B) Characterization of ANG I and ANG II extracted from brain stem of rats. (C and D) ANG I and ANG II in CSF after reaction with renin in vivo (C) or in vitro (D). The HPLC system consisted of two pumps (model 6000A), a model 660 solvent programmer, and a U6K injection system coupled to a variable wavelength UV-detector (model 450, Waters Associates). All separations were done on ODS-silica reversed phase columns (Bondapak C18, 300 by 4 mm, Waters Associates) with a particle size of 10 μm and two different gradient elution systems. (A) For the methanol gradient, 10 m*M* ammonium acetate buffer was adjusted to *p*H 4.5 or *p*H 5.4 with acetic acid, and methanol was increased linearly from 30 to 80 volumes per 100 volumes within 35 minutes. (B) For the isopropanol gradient, 10 m*M* triethylammonium-phosphate (TEAP) was adjusted to *p*H 3.0 with concentrated phosphoric acid. In a linear gradient, isopropanol concentration was increased from 5 to 40 volumes per 100 volumes within 45 minutes. Sample injection volumes varied between 100 and 950 μl; flow rate was 1.0 ml/minute and fractions were collected for 30 to 60 seconds into polyethylene tubes and subjected directly to radioimmunoassay. Blank controls for Sep-Pak purification and HPLC comprised the complete procedure as described but without sample application. Angiotensin was measured in the fractions by radioimmunoassay as described (*12*). Note the baseline separation of angiotensin peptides on the HPLC systems and the identity of ANG cleaved in vivo (C) and in vitro (D) by renin from CSF angiotensinogen and extracted from the brain (A and B) with synthetic [Ile^5]ANG I and [Ile^5]ANG II (arrows). The first peak of immunoreactivity with the ANG II antibody (B) is unidentified but may correspond to the ANG II-(4–8) pentapeptide fragment.