rounds the amplified sequence of EXT, there are several other repeats (Fig. 3) in this domain of all three variants of the satellite. No major changes occur near these repeated sequences.

Although bovine satellites 1.711a and 1.711b exhibit high homology to bovine satellites 1.706 and 1.715, respectively (*4*), their repeat units are larger. In both cases, the extra DNA is unrelated to the rest of the repeat unit and is described as inserted (*4*). In contrast, we describe an expanded satellite repeat unit that results from the amplification of an internal sequence. An inverted tetranucleotide repeat forms the boundaries of the amplified sequence in EXT, the only variant of the three studied to date that contains the amplification. At the site of amplification, the upstream arm of the inverted repeat is missing from the two other variants, RU and TRU.

Other sites of major sequence divergences in this crab satellite DNA also occur in association with domains of repeated DNA's (*5, 6*). Some of these are homopolymer tracts; others are homocopolymers and their variants. Still others contain long tracts of alternating purines and pyrimidines. All can be envisaged as having marked influences on secondary or tertiary structures, or both.

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Human Macrophages Armed with Murine Immunoglobulin G2a Antibodies to Tumors Destroy Human Cancer Cells

Abstract. *Macrophages isolated from tumor-bearing patients as well as cultured human monocytes express Fc receptors that cross-react strongly with murine immunoglobulins of the G2a but only slightly or not at all with the G1, G2b, or G3 subclasses. Such macrophages in the presence of murine immunoglobulin G2a monoclonal antibodies to tumors mediated the killing of tumor cells in vitro. These data suggest that monoclonal antibodies of the G2a subclass may be useful in the immunotherapy of human cancer.*

Murine monoclonal antibodies have been used successfully for the detection of cancer antigens in the blood of patients (*1–3*) or on the surface of tumor cells (*4, 5*). Such antibodies conjugated with radioactive labels have also been used to locate tumors (by imaging) in patients (*6*). Specific destruction of human tumor xenografts in nude mice (*7, 8*) was observed after inoculation of monoclonal antibodies of immunoglobulin G2a (IgG2a) isotypes only (*9, 10*), although all other isotypes were tested. We have also shown that tumor cells are destroyed as a result of the interaction of IgG2a monoclonal antibodies and mouse macrophages (*9*). Because tumor-specific monoclonal antibodies derived from humans are not yet available for immunotherapy we studied the interaction of murine immunoglobulins with human effector cells in vitro. We examined two basic phenomena: (i) whether human monocytes and macrophages express Fc receptors that cross-react with the various murine IgG subclass proteins and (ii) whether human monocytes and macrophages in the presence of murine monoclonal antibodies mediate lysis of human tumor cells.

We first established that human peripheral blood leukocytes would prevent

Table 1. Expression of Fc receptors for mouse immunoglobulins on human monocytes and macrophages. Human peripheral blood leukocytes (PBL) were prepared by Ficoll-Hypaque centrifugation. The PBL layer was washed twice in phosphate-buffered saline (PBS) containing 20 m*M* Hepes. The mononuclear cells were counted and standardized to appropriate concentrations in RPMI 1640 medium containing 20 m*M* Hepes and 0.1 percent autologous plasma or, alternatively, 10 percent fetal calf serum. Portions (1 ml) of PBL were distributed into 24-well tissue culture plates and allowed to adhere for 2 hours at 37°C in a 5 percent CO_2 atmosphere. The nonadherent cells were removed and the adherent monocytes were washed twice in RPMI 1640 medium. More than 97 percent of the adherent cells were monocytes, as determined by Wright's staining, nonspecific esterase activity, and latex phagocytosis. Macrophages from cancer patients were isolated from ascitic or pleural effusions after isolation of all cells by centrifugation. The cells were counted and the percentage of macrophages established by Wright's staining. Cells were standardized to appropriate concentrations in RPMI 1640 medium. Portions (1 ml) were distributed into 24-well plates and after 2 hours processed as above for selection of adherent cells. More than 90 percent of adherent cells were macrophages by the above criteria. Fc receptors were detected by incubating monocytes and macrophages with sheep or ox erythrocytes coated with mouse monoclonal antibodies to sheep red blood cells (established by W. C. Raschke, La Jolla Cancer Research Foundation, and received through ATCC) or to ox red blood cells (established by us). Red blood cells were coated with equivalent amounts of antibodies to saturate binding sites. More than five erythrocytes per cell were considered positive.

Source of human monocytes and macrophages	Donor	Days in culture	Percentage of cells expressing Fc receptors reacting with mouse immunoglobulins				
			G1	G2a	G2b	G3	M
Blood	J.K.	0 to 9	0	0	0	0	0
		10 to 14	0	90	0	0	0
		17 to 40	0	50	0	0	0
	M.K.	0 to 13	0	0	0	0	0
		14	0	20	0	0	0
		18	0	60	< 1	50	0
		34	0	90	0	0	0
	C.R.	0 to 20	0	0	0	0	0
		21	0	50	0	0	0
		29	0	90	0	0	0
Melanoma	WM369*	1	0	50	0	0	0
	WM373†	1	0	50	0	0	0
Colon carcinoma	J.R.†	1	0	55	0	0	0
		3	0	70	0	0	0
		7	0	90	< 5	0	0

*Isolated from pleural effusion. †Isolated from ascitic effusion.

the growth of human tumors in nude mice when tumor-specific IgG2a monoclonal antibodies were present: the effector cells alone or the antibody alone had no tumoricidal effect when mixed with tumor implants (data not shown). Thus, by analogy to the murine system (*9*), it was possible that human monocytes in that cell population were responsible for the destruction of tumor cells in the presence of mouse immunoglobulin.

The expression on human monocytes and macrophages of Fc receptors cross-reactive with murine immunoglobulins was examined by a rosetting assay (Table 1). Fc receptors cross-reactive with murine IgG subclass proteins were not detected on freshly cultured monocytes by this assay. However, after 10 to 20 days in culture, these cells expressed Fc receptors cross-reactive with murine IgG2a antibodies. The binding of other murine (non-IgG2a) immunoglobulins to cultured monocytes and macrophages was not detected by this assay. Macrophages obtained directly from pleural and abdominal effusions of cancer patients (Table 1) also expressed Fc receptors that cross-reacted with mouse IgG2a. Unlike Fc receptor expression on monocytes, Fc receptors for IgG2a on macrophages from tumor patients were readily detectable during the first 24 hours of tissue culture (Table 1). The interaction of murine IgG proteins with human monocytes and macrophages was subsequently analyzed in a radioimmunoassay (RIA). Table 2 shows that although all murine IgG subclass proteins showed poor binding to 1-day monocytes, there was a severalfold increase in the binding of murine IgG2a antibodies to monocytes cultured for 30 days relative to the IgG2a $F(ab')_2$ controls. All other (non-IgG2a) murine IgG proteins showed relatively poor binding to 30-day macrophages.

These experiments demonstrate that the interactions of murine IgG2a antibodies with human macrophages are mediated by the Fc portion of the immunoglobulin molecule. Although it is not known whether the Fc receptor involved in this binding is identical to the previously characterized Fc receptor of human monocytes (*11*), Fleit *et al.* (*12*), using a monoclonal antibody specifically reactive with Fc receptors on human neutrophils, have shown that human monocytes express a cross-reactive Fc receptor after cultivation in vitro for several days. Further, Eskewitz *et al.* (*13*) have shown in the murine system that activation of macrophages is associated with the selective enhancement of Fc receptor expression for IgG2a but not for IgG2b or IgG1 antibodies. It will be interesting to determine whether human macrophages possess multiple Fc receptors, similar to the murine system, and whether different receptors are involved

Table 2. Binding of ^{125}I-labeled monoclonal antibodies to 1-day monocytes and 30-day monocyte/macrophages. Antibodies were purified by protein A–Sepharose affinity chromatography as described by Ey *et al.* (*15*) and radiolabeled by using IODO-GEN (*16*) as described (*17*). Leukocytes were purified from peripheral blood by a Ficoll-Hypaque step gradient (*18*), and monocytes were separated from other mononuclear cells in a continuous Percoll gradient (*19*) as described (*20*). Monocytes were cultured overnight in a Teflon-coated beaker (Nalge Co.) in a 5 percent CO_2 atmosphere at 37°C in RPMI 1640 medium containing 10 percent fetal calf serum. Monocytes were then washed twice in PBS and resuspended in PBS containing 1.0 percent bovine serum albumin and 0.1 percent sodium azide (RIA buffer) and maintained at 4°C throughout the assay. Monocytes (2×10^5 cells in 50 μl of RIA buffer) and ^{125}I-labeled monoclonal antibodies (1×10^6 count/min in 50 μl of RIA buffer) were added to individual wells of 96-well polystyrene microtiter plates and incubated for 5 hours at 4°C with agitation. Cells were then washed three times in cold RIA buffer and the cell-associated radioactivity counted in a gamma counter. Thirty-day monocytes and macrophages were prepared by adherence selection of Ficoll-purified peripheral blood leukocytes in 24-well Linbro plates. Cells were then maintained in RPMI 1640 medium containing 10 percent fetal calf serum at 37°C in 5 percent CO_2. After 30 days in culture, the cells were washed twice in RIA buffer and the monolayers were incubated with ^{125}I-labeled monoclonal antibodies (1×10^6 count/min) in 150 μl of RIA buffer. Cells were incubated at 4°C for 5 hours with periodic agitation, washed three times in cold RIA buffer, solubilized in 5*N* NaOH, and counted in a gamma counter. All assays were performed in triplicate.

^{125}I-labeled antibody	Antibody subclass	^{125}I-labeled antibody bound (count/min)	
		One-day monocytes	Thirty-day monocyte/macrophages
JC4-Cl-4	G2a	6,281	44,164
JC4-Cl-4 $F(ab')_2$		2,798	2,618
17-1A	G2a	4,699	25,264
17-1A $F(ab')_2$		1,256	1,817
H36-1	G1	2,364	5,015
H37-79	G1	3,479	3,373
MOPC 141	G2b	6,544	4,160
H36-12	G2b	4,551	3,265
H36-5	G3	8,805	3,725
H36-17	G3	4,463	6,219

Table 3. Killing of human colon carcinoma cells by human effector cells mediated by mouse IgG2a monoclonal antibody. Monocytes and macrophages were isolated as described in Table 1. Human colon carcinoma cell line SW948 (*4*) was used as the target. SW948 target cells were labeled with methyl-[^{3}H]thymidine (5 μCi/10^6 cells; specific activity 25 Ci/mmole) and added to wells of 24-well plates with monocytes and macrophages at target-to-effector ratios of 1:10. This was followed by the addition of the monoclonal antibodies or tissue culture medium (controls). After 48 hours of incubation at 37°C in an atmosphere of 5 percent CO_2, the plates were removed and radioactivity release was determined in 100-μl portions of supernatant. Spontaneous and maximum release of radioactivity (in counts per minute) by targets was determined and the percentage of specific lysis was calculated as follows: (radioactivity of sample − spontaneous radioactivity)/(maximum radioactivity − spontaneous radioactivity) × 100. The differences in the two assay systems preclude a direct comparison of the actual percentages of cells expressing Fc receptor and the tumoricidal effects.

Source of human effector cells	Donor	Time in culture	Expression of Fc receptors for mouse IgG	Percentage of [^{3}H]thymidine release from SW948 colon carcinoma cells in the presence of IgG*				
				Antibody to tumor			Control	
				IgG2a	IgG2b	IgG1	IgG2a	None
PBL (monocytes)	C.R.	24 hours	Undetectable	19.5	8.5	8.7	3.3	3.3
PBL (monocyte/macrophages)	C.R.	29 days	IgG2a (90 percent)	86.7	7.8	13.1	9.5	14.6
PBL (monocyte/macrophages)	M.K.	34 days	IgG2a (90 percent)	85.7	11.9	33.5	16.9	9.4
Macrophages								
Colon carcinoma	J.R.	24 hours	IgG2a (50 percent)	15.2	4.3	3.1	1.4	4.3
(Ascites)		72 hours	IgG2a (70 percent)	17.7	4.3	10.4	12.3	7.9

*Monoclonal antibodies tested included (from left to right): IgG2a monoclonal antibody 17-1A which binds to SW948 cells in RIA; IgG2b monoclonal antibody 33-1 which binds to SW948 cells in RIA; IgG1 monoclonal antibody 19-9 which binds to SW949 cells in RIA; and IgG2a monoclonal antibody H24B5 to influenza virus, which does not bind to SW948 cells in RIA.

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*).

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

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.