

# 4F2 Monoclonal Antibody Recognizes a Surface Antigen on Spread Human Fibroblasts of Embryonic but Not of Adult Origin

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**ABSTRACT** The 4F2 monoclonal antibody (mAb) has been shown to recognize a 120-kilodalton glycoprotein expressed on the cell surface of human peripheral blood monocytes, activated (but not resting) T or B cells, and T and B lymphoblastoid cell lines. In this report we show that 4F2 mAb specifically binds to the surface of adherent human embryonic fibroblasts but fails to bind to normal adult fibroblasts. Moreover, 4F2 antigen was expressed on sarcoma-derived or SV40-transformed adult fibroblastic cells. Finally, addition of 4F2 mAb inhibited the growth of cultured HT-1080 fibrosarcoma cell line, but had no inhibitory effect on various embryonic and adult normal or transformed fibroblasts.

Several cell surface antigens present at the cell surface of human mononuclear cells have been recently defined by the use of monoclonal antibodies (mAbs)<sup>1</sup> (1). Some of these antigens are selectively expressed by T cell subsets with different functional activities or by cells at different stages of differentiation and/or maturation (2).

Although in some instances these surface antigens are selectively expressed by cells of the lymphoid lineage, several mAbs appear to recognize surface structures that may be expressed also by other cell types, including hemopoietic or neural cells (1, 3, 4).

A useful cell model system that has been extensively exploited for chromosomal, genetic, and biochemical analysis is represented by human fibroblasts of both embryonic and postnatal origin. Although a large amount of data are available on biological and biochemical properties, lifespan, growth rates, and functional patterns of embryonic and adult human fibroblasts (5–13), no surface markers have been available up till now that define different stages of fibroblast differentiation or activation. Thus, we tested a battery of mAbs on human fibroblastic cells of both embryonic and adult origin. The mAbs were selected on the basis of their reactivity with cell surface antigens expressed on mononuclear cells at different

differentiation and/or maturation stages and known to play a regulating role on different cell functions.

In the present report we show that the 4F2 mAb, which recognizes a 120-kilodalton glycoprotein (14) expressed on human peripheral blood monocytes, activated T or B cells, and T and B lymphoblastoid cell lines (14–16), binds to the cell surface of adherent fibroblasts of embryonic but not of adult origin. Furthermore, 4F2 antigen was detected on sarcoma-derived or SV40-transformed adult fibroblastic cells. Finally, addition of 4F2 mAb prevented the growth of cultured fibrosarcoma-derived HT-1080 cell line, but had no inhibitory effect on proliferation of a variety of embryonic or adult normal and transformed fibroblasts.

## MATERIALS AND METHODS

**Cell Lines:** Cell lines designated "964 lung" and "964 skin" were derived from the same embryo (kindly provided by Dr. J. Pontén, Uppsala University, Sweden); the cell lines WI-38 (7), IMR-90 (17), VA-13, MCR-5 (18), ICIG-7 (13), and ICIG-7-SV40, ICIG-8, ICIG-9 (the two latter derived from the same embryo), and the cloned somatic human-mouse hybrid MRC-5 × C11D (19) were kindly provided by Dr. A. Macieira-Coelho (Institut de Cancérologie et d'Immunologie, Villejuif, France). Embryonic cells were tested between the 10th and the 19th passage.

Adult cell lines designated AG-4151, AG-4353, AG-4445, and AG-4059 were purchased from the Human Genetic Mutant Cell Repository (Institute for Medical Research, Camden, NJ). AJ cells were obtained from Dr. L. Kopelovitch (Sloan-Kettering Memorial Cancer Center, NY) and NS and MVL cells from Dr. M. Marell (Ghent University, Belgium). HG and HG-SV40 were

<sup>1</sup> *Abbreviations used in this paper:* FCR, fibrin clot retraction; mAb(s), monoclonal antibody(s); MEM, Eagle's minimum essential medium.

obtained from Dr. A. Sarazin (Institut de Recherches Scientifiques sur le Cancer, Centre National de la Recherche Scientifique, Villejuif, France). Te-85 cell line was obtained from Dr. J. Rhim (National Institutes of Health, Bethesda, MD) and HT-1080 and RD cell lines were purchased from the American Type Culture Collection (Rockville, MD). Mns (12), Glen, DUP/N, and DUP/A were established in culture according to a previously described technique (12). Adult normal (HU-EYE) and SV40-transformed (HJ-EYE SV40) fibroblasts from human neuroretina were obtained from Dr. A. Benedetto (Virology Center, S. Camillo Hospital, Rome). Normal adult cells were tested between the 4th and the 12th passage. All the cell lines were found to be free of mycoplasma contamination. Cultures were maintained in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum and 16  $\mu$ g/ml gentamycin.

**Immunofluorescence:** Confluent cultures were trypsinized and the cells were resuspended in complete growth medium and seeded onto sterile glass coverslips layered on 60-mm Petri dishes filled with complete growth medium. 48 h later, the coverslips were washed three times in MEM and incubated at 4°C for 20 min with different mAbs (1:100 dilution of ascitic fluids in MEM) including 4F2, 3A1 (reacting with 85% human peripheral blood T cells) (20), D1/12, D4/22, 9/22, and PTF (all directed against HLA-DR molecules) (21, 22), and BT3/4 (which recognizes the HLA-DR-like DC1 supertypic specificity) (22). After being extensively washed in MEM, the coverslips were incubated with a fluorescein isothiocyanate-conjugated goat anti-mouse Ig. After 20 min at 4°C, the coverslips were washed three times in MEM and mounted in 90% glycerol in PBS. Controls were performed by incubating with the second reagent only.

**Addition of 4F2 mAb and Evaluation of Cell Spreading, Fibrin Clot Retraction (FCR), and Growth Rates:** To evaluate the effect of 4F2 mAb on cell spreading and FCR, confluent ICIG-7 and HT-1080 cultures were trypsinized, resuspended in growth medium, and centrifuged at 1,000 rpm for 10 min. The cell pellets were resuspended in Tyrode solution with or without 4F2 mAb (1:100, 50  $\mu$ l  $\times$  10<sup>6</sup> cells) and incubated at 4°C for 20 min.

Cell suspensions were then scored visually by indirect immunofluorescence assays. The cell suspensions were then seeded onto plastic Petri dishes with or without a fibrin gel layer (5  $\times$  10<sup>4</sup> cells per 30-mm dish) or tested for FCR according to previously described techniques (24, 25).

To evaluate the effect of 4F2 mAb on the growth rate of 4F2<sup>+</sup> cell lines, confluent ICIG-7, ICIG-7-SV40, WI-38, VA-13, HG-SV40, TE-85, and HT-1080 cells were trypsinized and resuspended in MEM supplemented with 10% heat-inactivated fetal bovine serum and 1:100 dilutions of 4F2 mAb or ascitic fluid obtained by injection of the original myeloma (P3  $\times$  63/Ag8) in the syngenic mice (14). Cells were then seeded at 7  $\times$  10<sup>4</sup> cells per Petri dish and at daily intervals cells of two cultures in each group were recovered by trypsinization and counted with an electronic Coulter counter. Medium with or without 4F2 mAb was substituted every 24 h.

## RESULTS

In a preliminary screening, the embryonic ICIG-7 and the

adult MVL cell lines were tested for reactivity with the panel of mAbs indicated in Materials and Methods. None of the mAbs analyzed reacted with adult or embryonic fibroblasts, with the remarkable exception of the 4F2 mAb which selectively stained 100% of embryonic cells. (Fig. 1A). To further investigate the distribution pattern of 4F2 antigen expression on human fibroblastic cells of different origins, several cell lines derived from embryonic, adult, or malignant tissues were tested by indirect immunofluorescence.

As shown in Table I, all the embryonic cell lines analyzed bound 4F2 mAb. No substantial difference in 4F2 expression was detected among cell lines derived either from different embryonic tissues or from embryos of different ages. The two SV40-transformed cell lines VA-13 and ICIG-7-SV40 were 4F2-positive in a similar fashion as the nontransformed parental cell lines (WI-38 and ICIG-7).

We further analyzed the 4F2 expression on a cloned somatic cell hybrid obtained by fusing human embryonic 4F2-positive fibroblasts with a 4F2-negative established murine cell line; 100% of the cells of this hybrid, designated MRC-5  $\times$  C11D, displayed bright fluorescence. However, as shown in the right column of Table I, all the fibroblastic cell lines derived from adult donors were 4F2 negative (Fig. 1B).

### 4F2 Antigen Expression on Neoplastic Cells

As it is well established that neoplastic cells may express embryonic antigens, we investigated whether 4F2 antigen would be expressed by sarcoma-derived adult human cell line or by adult fibroblasts transformed by SV40. To this end, we analyzed the 4F2 expression on three SV40-transformed and three sarcoma-derived adult cell lines. All the six neoplastic lines analyzed displayed a strong reactivity (Fig. 1C), while the normal counterpart of SV40-transformed cell lines were 4F2-negative. It is noteworthy that in the initial phases of SV40 transformation, only a fraction of the cultured cells expressed the 4F2 antigen; moreover, similar percentages of cells expressed the nuclear viral T antigen as assessed by indirect immunofluorescence. In addition, both 4F2 and T antigen showed a parallel increase during culture (data not shown).

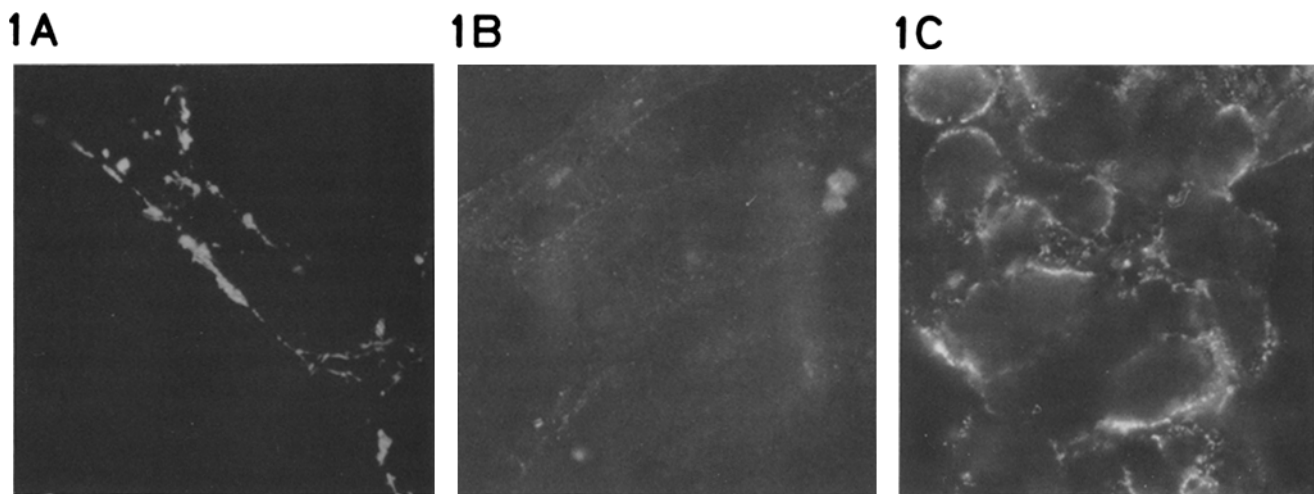


FIGURE 1 Immunofluorescent staining of human fibroblasts, spared onto glass coverslips with the 4F2 mAb. (A) Human embryonic cells (ICIG-7) show a bright, dotted fluorescence on the cell membrane. On the contrary, (B) human adult fibroblasts (DUP/N) are not stained. However, after SV40 transformation (DUP/N-SV40), they appear brightly stained by the 4F2 mAb (C).  $\times$  800.

TABLE I  
Binding of 4F2 mAb to Embryonic, Adult, or Malignant Human Fibroblastic Cells

Embryo fibroblasts	Embryo's age	4F2 Binding	Adult fibroblasts	Donor's age	4F2 Binding
	<i>mo</i>			<i>yr</i>	
IMR-90 Lung	4	+	Normal	52	-
964 Lung	not known	+	GLEN Bone-marrow	54	-
964 Skin	not known	+	HAL-14 Lung	52	-
ICIG-7 Lung	5	+	DUP-A Aponevrosis	30	-
WI-38 Lung	3	+	NS Skin	29	-
ICIG-8 Skin	4	+	MVL Skin	30	-
ICIG-9 Lung	4	+	AG 4151 Skin	59	-
MRC-5 Lung	3½	+	AG 4353 Skin	70	-
MRC-5 × C11D (somatic human × mouse)		+	AG4445 Skin	88	-
ICIG-7 SV40		+	AG 4065 Skin	96	-
VA-13 (WI-38 SV40)		+	AG 4055 Skin	29	-
			NMS-1 Skin	24	-
			AJ Skin	52	-
			DUP-N Fibroma	17	-
			Hu-EYE Neuroretina		-
			HG Skin		
			Neoplastic		
			DUPN-SV40		+
			HG-SV40		+
			HU-EYE-SV40		+
			HT-1080 Fibrosarcoma		+
			TE-85 Osteosarcoma		+
			RD Rabdomyosarcoma		+

TABLE II  
Effect of the Exposure to 4F2 Monoclonal Antibody

	ICIG-7	ICIG-7 + 4F2	HT1080	HT1080 + 4F2
Spreading efficiency				
On plastic*	+++	+++	+++	+++
Onto fibrin*	++++	++++	++++	++++
FCR	100%	100%	30%	30%
Number of population doublings 96 h after seeding*	3	3	4.5	0.5

\* 5 h after seeding, cultures were fixed by addition of glutaraldehyde to growth medium (final concentration 5%). Thereafter 10 fields were scored at random in three Petri dishes of each group for the degree of spreading and the number of spread cells. The spreading efficiency is expressed in arbitrary units ranging from + to ++++.

\* Cells were initially seeded at  $7 \times 10^4$  cells per Petri dish.

### Effect of 4F2 mAb on Cell Surface-related Functions

The growth of fibroblastic cells *in vitro* is dependent on their ability to attach to surfaces, spread, contact, and move. Since all of these processes are intimately connected with cell division and are therefore likely to be controlled by cell surface structures, we investigated the possible involvement of the 4F2 antigen in these cell activities. Toward this end, we analyzed the effect of 4F2 mAb on (a) cell attachment and spreading on different surfaces, (b) cellular contractility, which was measured as FCR activity, and (c) growth rates of 4F2-positive fibroblastic cells. In experiments designed to measure the effect of 4F2 mAb on cell spreading efficiency or FCR activity, cells were detached by trypsin treatment and resuspended in Tyrode solution. Trypsin did not affect the 4F2 antigen expression as assessed by indirect immunofluorescence (data not shown). As summarized in Table II, addition of 4F2 mAb did not affect the spreading efficiency nor the FCR activity of both embryonic ICIG-7 fibroblasts and HT-1080 fibrosarcoma cells; the observation of a reduced FCR

activity in sarcoma cells (as compared with normal cells) is in agreement with previous reports (24, 25).

In another series of experiments, we studied the effect of addition of saturating amounts of 4F2 mAb on the growth rates of different 4F2-positive cell lines. It was found that 4F2 mAb had no effect on the proliferation of ICIG-7, ICIG-7-SV40, WI-38, VA-13, HG-SV40, and Te-85 cells, but had a sharp inhibitory activity on the growth of the HT-1080 fibrosarcoma-derived cell line. A representative experiment is shown in Fig. 2. Saturating amounts of 4F2 mAb had no effect on ICIG-7 (Fig. 2A), but sharply inhibited the proliferation of HT-1080 cells (Fig. 2B). Similar results were obtained in three additional experiments. The inhibitory effect of 4F2 mAb on HT-1080 cell proliferation could not be simply explained by a cytotoxic effect of the antibody for the following reasons. First fetal bovine serum supplements were heat inactivated; second, the mAb did not affect the growth of other 4F2-positive cell lines, nor did it have a cytotoxic effect on monocytes or activated T and B cells in the absence of complement; and finally, 90% HT-1080 cells, culture for 48 h in the presence of 4F2 mAb, were viable, as assessed by trypan blue exclusion test.

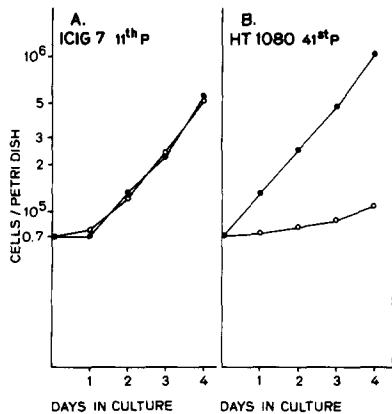


FIGURE 2 Semilogarithmic plots of cell numbers in ICIG-7 (A) and HT-1080 (B) cultures exposed (O) or not exposed (●) to 4F2 monoclonal antibody.

## DISCUSSION

Our present studies show that the 4F2 mAb originally raised against human mononuclear cells (14–16) can be used to identify transformed fibroblastic cells derived from adult donors as well as normal fibroblasts at an early stage of development. Moreover we provide evidence that the 4F2 mAb inhibits the growth of a 4F2-positive human fibrosarcoma cell line. These data are of particular interest because no surface markers have thus far been available that allow one to distinguish between embryonic and adult human fibroblasts. The difference observed in 4F2 antigen expression on cells at different stages of differentiation may be important in view of previous reports that suggested the existence of a relationship between changes in the moieties of certain surface glycoproteins and growth decline in old versus young cell cultures (27). It is likely that several surface molecules may be involved in the functional activities of the cells themselves. For example, it has been shown that mAbs specific for HLA-DR molecules inhibited the interleukin-2-dependent proliferation of human T cells and T cell clones (21). In addition, it has been reported that 4F2 mAb itself partially inhibited the mitogenic response of T cells to phytohemagglutinin and concanavalin A (14). Our present data suggest that 4F2 surface molecules are not involved in spreading and contractile activity of normal and tumoral fibroblasts, but they may play a role in the growth regulation of human fibrosarcoma cells. The differential effect of 4F2 mAb on the growth of 4F2-positive fibrosarcoma cells versus 4F2-positive embryonic fibroblasts could be related to the existence of different molecular forms of the 4F2 antigen. In this context, Hemler and Strominger recently demonstrated the existence of different molecular forms of 4F2 antigen on human T and B lymphoblastoid cell lines (28). Thus, under reducing conditions, the 4F2 antigen appeared to be composed of a sialylated heavy subunit of 85,000 daltons on T cells and 93,000 daltons on B cells and of a light subunit of 41,000 daltons common to T and B cells. According to preliminary data, HT 1080 fibrosarcoma cells express two types of heavy subunits, as assessed by mono- and bidimensional gel electrophoresis; in contrast, embryonic cells seem to express predominantly one type of heavy chain (Azzarone, B., P. Eid, Y. Malpieces, H. Suarez, and A. Fauci, manuscript in preparation). It is of interest that a cloned somatic hybrid obtained by fusing the 4F2-positive MRC-5 human embryonic lung fibroblastic cells with the 4F2-negative C3H-C11D murine cells expressed the 4F2 antigen, although only few human chromosomes could be iden-

tified in this hybrid (19). Our data are in agreement with a recent report by Messer-Peters et al., who showed that the 4F2 antigen maps to chromosome 11 (29).

Experiments are presently in progress to establish whether SV40-induced transformation of adult fibroblasts causes a de novo expression of 4F2 antigen as previously shown for human T cells following activation in mixed lymphocyte culture (1, 14) or whether it only causes unmasking of an internal antigenic determinant. In addition, we are presently investigating whether differential effects on the growth rates of different 4F2-positive cell lines are associated with the presence of different molecular forms of 4F2 antigen. Hopefully, these studies will provide useful tools for a more accurate dissection of the complex events associated with cellular differentiation and/or transformation.

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