MACROPHAGE-MELANOMA CELL HETEROKARYONS

IV. Unmasking the Macrophage-Specific Membrane Receptor*

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The plasma membrane of the mouse peritoneal macrophage has specific receptors which enable the cell to ingest antibody-coated sheep red cells and is also rich in a divalent cation-dependent adenosine triphosphatase (ATPase) activity (1, 2). By fusing macrophages with a strain of mouse melanoma cells which lacks these receptors, one could not only trace the fate of the macrophage membrane in the heterokaryons, but also hope to learn about the expression of cell-specific traits in animal cells (3).

Macrophage homokaryons obtained by Sendai virus-induced cell fusion continued to exhibit active phagocytosis of sensitized erythrocytes. The phagocytic receptor could also be detected in heterokaryons shortly after fusion, but was progressively lost over the next 12–24 hr. The ATPase activity disappeared from heterokaryons as well, and in addition, seemed to spread out over the cell surface.

Several possible mechanisms could account for these observations. These include mixing of membrane components resulting in dilution and loss of function; endo- or exocytosis without further synthesis; and masking of the macrophage membrane markers, either by serum factors or by the heterokaryon itself. We report experiments to distinguish among these hypotheses.

Materials and Methods

Cell Culture and Cell Fusion Technique.—Carried out as described (3). The macrophage phagocytic receptor was assayed with minor modifications of our previous procedure. Cover slip preparations were exposed at room temperature for 15 min to a 0.1% suspension of sheep red blood cells (SRBC)[1] which had been coated with a $\frac{1}{3000}$ dilution of a rabbit anti-sheep

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[1] *Abbreviations used in this paper:* AbSRBC, antibody-coated sheep red blood cells; BGG, bovine gamma globulin; BSA, bovine serum albumin; EAT, Ehrlich ascites tumor cells; IgG, immunoglobulin G; IPT-FCS, immunoprecipitin-tested fetal calf serum; NBCS, newborn calf serum; 199 M, medium 199 + 10% NBCS; SRBC, sheep red blood cells; STI, soybean trypsin inhibitor.

red cell antiserum which contained mainly immunoglobulin G (IgG) antibody. Assays were performed in the absence of complement. The cover slips were washed vigorously and incubated for 30 min at 37°C in medium 199 (Microbiological Associates, Inc., Bethesda, Md.) + 10% newborn calf serum (NBCS) (hereafter 199 M). Cells were fixed with glutaraldehyde and stained with 0.1% toluidine blue in 30% methanol. Ingested and attached red cells stain distinctively by this procedure. 100 cells were scored at random except where noted to the contrary. The mean number of red cells ingested per macrophage was used as an index of ingestion. In the present experiments the failure to ingest red cells was associated with a failure in attachment.

Reagents.—The materials used in these experiments were purchased from the following sources: NBCS and immunoprecipitin-tested fetal calf serum (IPT-FCS), Grand Island Biological Co., Grand Island, N.Y.; fraction V and bovine serum albumin (BSA), crystalline, Armour Pharmaceutical Co., Kankakee, Ill.; Pronase, Calbiochem, Los Angeles, Calif.; disodium ethylenediaminetetraacetate (EDTA), Fisher Scientific Co., Fair Lawn, N.J.; bovine gamma globulin (BGG), Pentex Biochemical, Kankakee, Ill.; neuraminidase, cholera vibrio, 500 units/ml, Behringwerke AG, Marburg-Lahn, West Germany, supplied by Certified Blood Products, New York; neuraminidase (*Clostridium perfringens*, 1 unit/mg), soybean trypsin inhibitor (STI) (twice crystalline), and bovine submaxillary mucin, Sigma Chemical Co., St. Louis, Mo.; trypsin (twice crystalline), chymotrypsin, papain, β-glucosidase (3.1 units/mg), and β-galactosidase (320 units/mg), Worthington Biochemical Corp., Freehold, N.J.

Enzyme and Chemical Treatment of Heterokaryons.—Fused preparations were cultivated in medium 199 + 20% NBCS for 1–2 days till most phagocytic receptor activity had been lost. The cells were washed two to four times in 199 and then treated with a particular reagent for 30 min at 37°C. The reagent was removed and the cells then handled according to the morphologic effect of each form of treatment. The cells in this fusion system react differently to trypsin: melanoma cells round up and become detached; macrophages spread and resist detachment; heterokaryons show intermediate behavior (3). If melanoma cells and heterokaryons were not detached by treatment, the preparations were washed two to four times in 199 and assayed for phagocytic function; if detachment did occur the cells were either incubated *in situ* in 199 M for 30–90 min, until they reattached, or replated on new cover slips. In the latter case the detached cells were collected by gentle pipetting in 199 M, spun at 1000 rpm for 5 min, and suspended in a small volume of 199 M for replating. Receptor activity was measured after 30–90 min.

Appropriate controls were set up for each treatment. A trypsin solution was heated for 10 min at 100°C to inactivate the enzyme. Neuraminidase activity was assayed in 199 and in 0.1 M acetate buffer, pH 5.0, with bovine submaxillary mucin as substrate. Free sialic acid was measured by the procedure of Warren (4).

25 1:1 heterokaryons and 100 macrophages were scored in duplicate for attachment and ingestion of antibody-coated sheep red blood cells (AbSRBC). Melanoma cells were always negative.

The Role of Serum Factors in Receptor Loss.—Heterokaryons were prepared after cultivating macrophages in 199 + 15% IPT-FCS for 1 day. 1 hr after fusion cover slips were placed in either 199 + 15% IPT-FCS, 199 + 1% BSA, or 199 + 1% fraction V. Receptor function was measured 4 and 20 hr after fusion. Some preparations were assayed after trypsin treatment, 100 μg/ml for 30 min at 37°C.

In another set of experiments the fused cells were incubated in nonspecific BGG. 1 hr after fusion groups of cover slips were placed in 199 plus one of the following: 20% NBCS, 20% IPT-FCS, 20% IPT-FCS + 1 mg/ml BGG, or 20% IPT-FCS + 10 mg/ml BGG. Receptor

activity was assayed 2, 9, and 21 hr after fusion. Before the assay the preparations were washed four times with 199 to remove free BGG.

The Role of Protein and RNA Synthesis in Receptor Loss.—Cycloheximide and bromotubercidin were used to inhibit protein and RNA synthesis, respectively (5, 6).

Cells were exposed to cycloheximide, 5 μg/ml, from 1 hr after fusion. After 4–8 hr treatment the cover slips were washed three times in 199 and phagocytic function assayed immediately, or at a later time.

Treatment with bromotubercidin, 5 μg/ml, was started before or after fusion. Bromotubercidin was present during fusion when necessary.

Irradiation with Ultraviolet Light before Fusion.—Macrophages and melanoma cells were cultivated in 60-mm Falcon plastic tissue culture dishes (Falcon Plastics, Los Angeles, Calif.) for 1 day. The cells were washed twice, covered with 1 ml of 199, and irradiated for 10–90 sec at a distance of 20 cm with a Sylvania germicidal lamp (G 15T8). After irradiation the cells were washed and incubated in 199 M. The preparations were fused 1–2 hr after ultraviolet light treatment and then incubated in medium 199 + 20% NBCS until ready for assay of their receptor activity.

Macrophage Fusion with Different Cells.—Red blood cells were obtained from 12-day old chick embryos (5). Fibroblast primary cultures were prepared from chick embryos (7). Ehrlich ascites tumor (EAT) cells were provided by Dr. E. Borenfreund, Sloan-Kettering Institute, New York. The ascites tumor was passaged in NCS mice and harvested 4–5 days after inoculation. 1×10^4–1×10^6 of these cells were pipetted onto macrophage monolayers. After 1 hr the preparations were treated with 1000 hemagglutinating units of ultraviolet-inactivated Sendai virus (3). The cover slips were washed 30–60 min later and placed in medium 199 + 20% NBCS. Phagocytic function was assayed at intervals.

Many of the chick red cells and most of the EAT cells which did not fuse with macrophages were washed away subsequently. The macrophages ingested the other unfused red cells so that pure populations of macrophages and heterokaryons remained after 1 day. The EAT cells did not attach to glass or grow in vitro unless they had fused with a macrophage. All heterokaryons were identified on the basis of nuclear morphology.

RESULTS

The Recovery of Phagocytic Function by Heterokaryons.—Heterokaryons which lacked phagocytic receptor activity after cultivation for 2 days immediately recovered full receptor function upon treatment with trypsin (Figs. 1, 3 *a*). If such heterokaryons were then cultivated further in fresh serum-containing medium, the receptor once more became undetectable. The extent of recovery of receptor activity depended on the concentration of trypsin employed (Table I). Full recovery occurred after treatment with 1–10 μg/ml trypsin for 30 min at 37°C. These changes in heterokaryon receptor activity were brought about without change in phagocytosis by unfused macrophages present in the same preparation.

Control experiments were performed to exclude a sampling artifact, in which trypsin treatment could have selected a population of heterokaryons rich in macrophage receptor activity. Trypsin treatment was limited to brief periods at room temperature (Table II). Receptor activity reappeared after

treatment with 2 μg/ml trypsin for 6 min, at which time heterokaryons had not yet become detached from glass. In another experiment all the heterokaryons were deliberately detached from cover slips by treatment with 100 μg/ml trypsin for 30 min at 37°C and then replated on a fresh cover slip. This population of heterokaryons also recovered full receptor activity.

Trypsin treatment did not alter the specificity of the interaction between

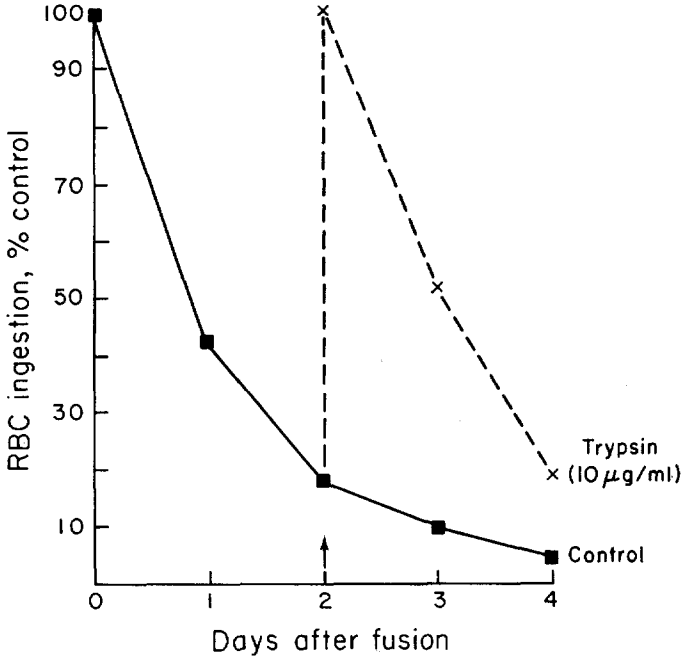

FIG. 1. Recovery of the phagocytic receptor in heterokaryons. Trypsin treatment 2 days after fusion (arrow).

antibody-coated red cells and macrophage receptor. Melanoma cells did not bind any AbSRBC after trypsinization and antibody on the red cell was obligatory for attachment as well as ingestion.

1–2-day old heterokaryons lacking demonstrable phagocytic activity were subjected to a variety of chemical and enzymatic treatments, summarized in Table III. These experiments demonstrated clearly that proteolysis was necessary to recover receptor activity. Heat inactivation of the trypsin or the presence of soybean trypsin inhibitor prevented its effect. Different proteolytic enzymes like pronase, chymotrypsin, and papain were as effective as trypsin. Treatment with EDTA, on the other hand, was ineffective at concentrations which detached heterokaryons from the glass. Neuraminidase treatment was

TABLE I

*The Effect of Trypsin Treatment on the Ingestion of Antibody-Coated SRBC**

Treatment (μg/ml trypsin)	Time after treatment	1:1 Heterokaryons	Macrophages	Ratio heterokaryon: macrophage × 100
	hr			
Control	−24	3.2	6.6	50
		2.7	6.6	41
	0.5	2.4	8.2	29
		1.2	7.1	17
	3	1.6	6.7	24
	24	1.1	6.3	17
		1.0	5.7	18
	48	0.55	9.3	5
10	0.5	5.4	5.3	102
		5.9	6.6	90
	3	5.8	5.6	104
	24	2.2	4.8	46
		3.8	6.5	58
	48	0.80	6.0	13
		0.36	5.4	7
1	0.5	6.1	6.6	93
		4.9	6.5	76
	3	5.0	7.0	70
		4.5	6.1	75
	24	3.5	6.5	54
		2.8	6.8	41
	48	0.50	7.0	7
0.1	0.5	3.2	6.0	53
		2.1	6.1	34
	3	2.3	6.8	34
	24	2.0	6.8	30
		1.8	7.4	24

* Trypsin treatment 2 days after fusion for 30 min at 37°C. Incubated in medium 199 + 15% NBCS after treatment. SRBC coated with 1/2000 antiserum. Assayed *in situ*, in duplicate.

also ineffective. Control experiments confirmed that when the enzyme was used in medium 199 it retained 77% of its activity in acetate buffer, pH 5.0. These findings gave rise to the hypothesis that proteolytic treatment removed or altered surface protein(s) which had masked receptor activity and that the receptor could again become masked during subsequent in vitro cultivation.

The Role of Serum Factors in Blocking the Heterokaryon Phagocytic Receptor.—Masking proteins could have been adsorbed directly from the serum in the culture medium or synthesized by the heterokaryon itself. In the former event the adsorbed proteins could be nonspecific gamma globulins or some other serum proteins.

TABLE II

*In Situ Unmasking of 1:1 Heterokaryons after Brief Trypsin Treatment**

Concentration	Duration of treatment	Mean No. AbSRBC ingested
μg/ml	*Min*	
5	2	0.2
	4	0.9
	6	3.3
	8	6.3
2	2	0.8
	4	0.8
	6	4.4
	8	3.2

* Trypsin treatment 2 days after fusion at room temperature. Washed three times and assayed immediately.

Cells were therefore cultivated in the absence of gamma globulin or placed in a medium in which BSA was the only protein. In all cases (Table IV *a*) receptor activity dropped to 15–30% of the initial activity in 20 hr and this effect could be reversed by treatment with trypsin.

In other experiments nonspecific BGG was added to the culture medium after fusion (Table IV *b*). This experiment showed that the rate of disappearance of the phagocytic receptor could be slightly accelerated by adding large quantities of BGG (10 mg/ml) to the medium, but was not a prerequisite for its loss. It was therefore concluded that serum proteins were not the major cause of the blocked receptors.

The Effect of Inhibitors on Receptor Disappearance.—If the heterokaryon made the proteins which blocked the phagocytic receptor, could this process be prevented?

An experiment in which cycloheximide was used to block protein synthesis after fusion is illustrated in Fig. 2. Unlike the control preparation in which receptor activity was lost, the cyclohexmide-treated heterokaryons maintained full phagocytic activity. Receptor activity in the unfused macrophages was also unaffected by cycloheximide treatment. When the block in protein syn-

TABLE III

*The Effect of Chemical and Enzymatic Treatments on Masked Heterokaryons**

Treatment	Ingestion of AbSRBC		Ratio heterokaryons: macrophages × 100
	1:1 Heterokaryons‡	Macrophages§	
Nil	0.60	6.3	10
Trypsin (5 μg/ml)	4.3	5.5	78
Heat inactivated	0.78	7.8	10
+12 μg/ml STI	0.52	9.6	5
+6 μg/ml STI	1.3	7.0	19
Pronase (10 μg/ml)	9.8	8.8	111
Chymotrypsin (10 μg/ml)	5.3	6.5	82
Papain control (medium 199 + 0.01 M cysteine HCl)	1.5	6.5	23
Papain (10 μg/ml) (in medium 199 + 0.01 M cysteine HCl)	6.0	6.1	99
EDTA control (Ca, Mg-free buffer)	0.95	6.0	18
EDTA 5.4×10^{-4} M	1.2	9.9	12
	1.1	7.3	11
Neuraminidase, *C. vibrio* (100 units/ml)	1.8	7.9	23
C. perfringens (1 unit/ml)	1.1	N.D.	—
β-Galactosidase (10 μg/ml)	0.85	6.2	14
β-Glucosidase (500 μg/ml)	0.80	7.2	11

* Fused preparations cultivated in medium 199 + 20% NBCS for 1 day, washed twice, and treated for 30 min at 37°C with appropriate reagent. Assayed, *in situ*, 0–90 min after treatment.

‡ Mean for 25 heterokaryons.

§ Mean for 100 macrophages.

TABLE IV

(a) The Masking Reaction in the Absence of Serum Factors

Medium	4 Hr after fusion		Uptake of AbSRBC by 1:1 heterokaryons 20 hr after fusion			
			No trypsin		Trypsin	
	Mean	SE	Mean	SE	Mean	SE
199 + 15% IPT-FCS	13	1.5	3.0	0.57	12	2.0
199 + 1% fraction V	15	1.5	2.1	0.54	16	3.3
199 + 1% BSA	14	1.8	4.3	0.90	13	2.0

(b) The Effect of Incubation in Nonspecific BGG on the Ingestion of AbSRBC by 1:1 Heterokaryons

Time after fusion	NBCS Control	IPT-FCS	IPT-FCS +1 mg/ml BGG	IPT-FCS +10 mg/ml BGG
hr				
2	7.9	—	—	—
9	1.8	4.0	3.5	1.5
21	0.64	1.4	0.75	—

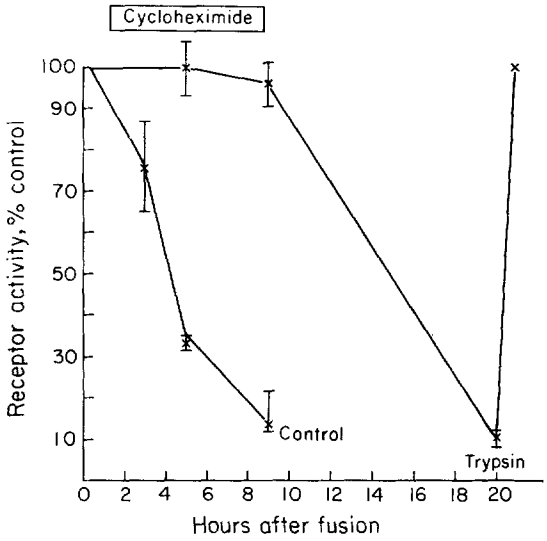

FIG. 2. The requirement for protein synthesis to mask the phagocytic receptor in heterokaryons.

thesis was reversed by washing out the drug, receptor activity was lost subsequently and could again be uncovered with trypsin.

In another experiment cells were treated with cycloheximide for different periods of time and the reversible inhibition of masking studied in more detail

(Table V). Receptor activity could be maintained for 8 hr in the presence of cycloheximide. Removal of the drug after 4 or 6 hr resulted in loss of receptor activity at a rate similar to that of the untreated control, with a half-life of the order of 3 hr. The cycloheximide effect was still reversible after an 8 hr treatment, but with a 2 hr lag period.

Some unfused macrophages are killed by 6–8 hr continuous exposure to cycloheximide, but heterokaryons and melanoma cells are able to survive. Even an 8 hr treatment with cycloheximide does not interfere with phagocytosis in active heterokaryons (Fig. 3 *b*) or surviving macrophages.

Preparations were also treated with cycloheximide for 6–8 hr, starting 20 hr after fusion, to see if the receptor could be uncovered by inhibiting protein

TABLE V

*The Reversible Inhibition of Masking by Cycloheximide**

Time after fusion	Mean No. AbSRBC ingested by 1:1 heterokaryons			
	Control	CH 1–5‡	CH 1–7	CH 1–9
hr				
1	5.6	—	—	—
5	3.4	5.0	—	—
7	3.0	3.4	6.3	—
9	2.1	1.7	4.2	6.4
11	0.9	1.3	2.5	7.6
22	—	—	—	2.2

* Groups of cover slips treated with 5 μg/ml cycloheximide for 4, 6, or 8 hr starting 1 hr after fusion. The cells were then washed three times, incubated in medium 199 + 20% NBCS, and assayed at different times.

‡ Cycloheximide treatment 1–5 hr after fusion.

synthesis after the period of masking. Receptor activity remained masked in this experiment. This meant that cycloheximide treatment, per se, did not bring about increased receptor activity in the heterokaryons, but that protein synthesis had to be blocked during the period immediately after fusion.

The role of RNA synthesis in masking the receptor was investigated next, using bromoturbercidin as a reversible inhibitor (Table VI). When heterokaryons were treated with bromotubercidin 1–9 hr after fusion, there was no effect on masking, whereas cycloheximide treatment during the same period prevented masking (Table VI *a*). If, however, bromotubercidin treatment was started 2 hr before fusion, masking was effectively prevented (Table VI *b*). This inhibition of masking was reversible after 12 hr treatment (Table VI *c*).

Ultraviolet Irradiation of Cells before Fusion.—Melanoma cells and macrophages were irradiated with ultraviolet light before fusion to separate the contribution of each heterokaryon to the masking reaction. The effect of such

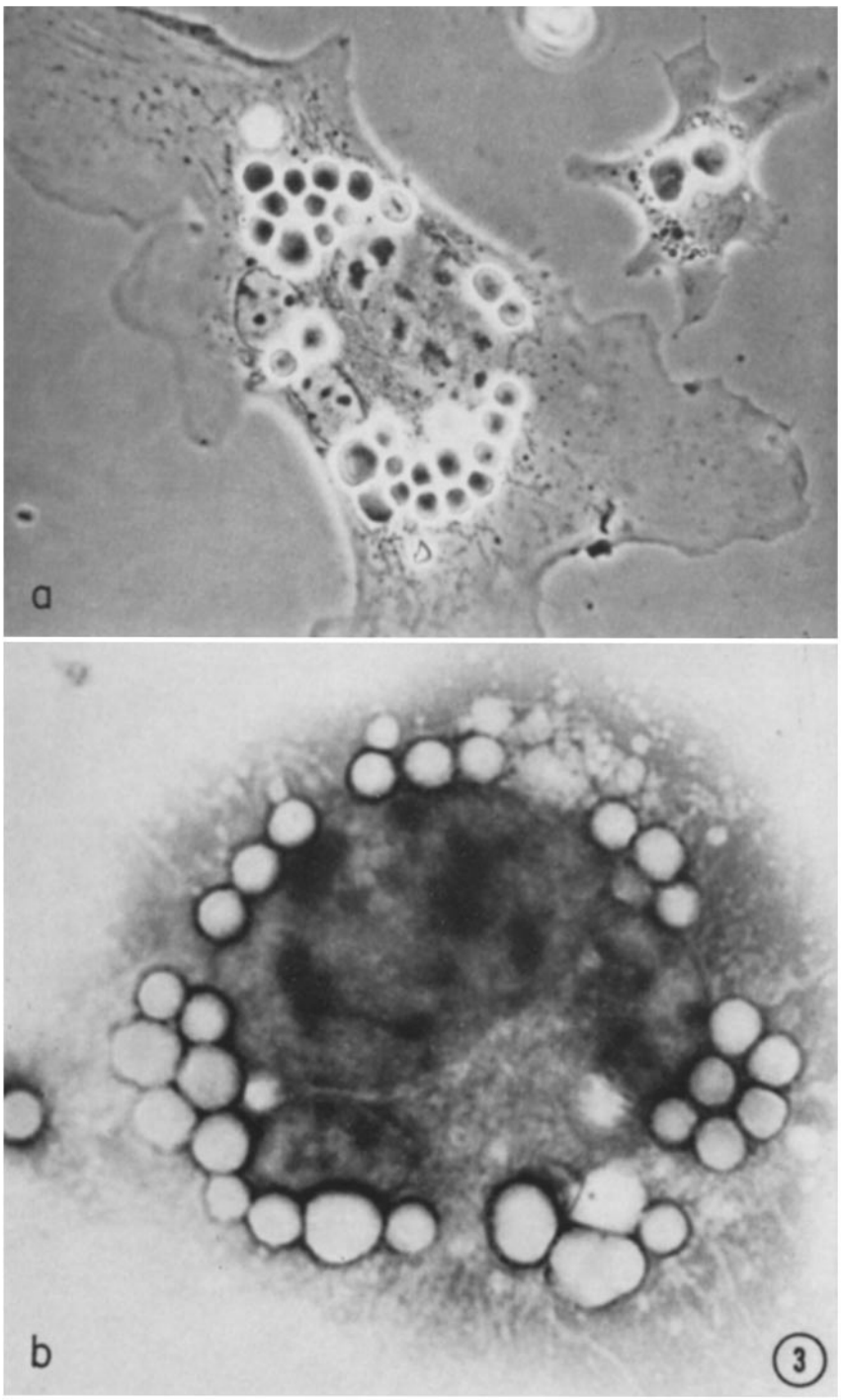

FIG. 3. Uptake of AbSRBC by heterokaryons under a variety of conditions. (*a*) A 2 day old heterokaryon ingests numerous red cells after treatment with trypsin to unmask the phagocytic receptor. Phase-contrast, × 800. (*b*) A 9 hr old heterokaryon displays active ingestion of red cells after 8 hr treatment with cycloheximide to prevent masking. Heterokaryons are typically well spread after such treatment. Stained preparation. × 1500.

treatment is shown in Table VII. Melanoma cell pretreatment preserved phagocytic function in heterokaryons, whereas macrophage pretreatment had no effect on receptor loss.

Both cell fusion and phagocytic activity were unaffected by ultraviolet pretreatment. Similar results were obtained after pretreating the cells for longer

TABLE VI

The Requirement for RNA Synthesis to Mask the Fc Receptor in 1:1 Heterokaryons

(*a*) Bromotubercidin (BT) treatment 1–9 hr after fusion

Time after fusion	Ingestion of AbSRBC		
	Control	Cycloheximide*	Bromotubercidin treatment
hr			
2.5	9.0	—	—
5	3.2	10.2	4.7
9	1.7	8.1	2.8

(*b*) Continuous bromotubercidin treatment started before, or after, fusion

Time after fusion	Ingestion of AbSRBC			
	Control	BT started 2 hr after fusion	BT started 0.5 hr after fusion	BT started 2 hr before fusion
hr				
2	7.0	—	—	9.7
7	1.8	2.8	3.8	8.2

(*c*) The reversible inhibition of masking by bromotubercidin treatment

Time after fusion	Ingestion of AbSRBC		
	Control	Bromotubercidin treatment (started 5 hr before fusion)	
hr			
2	5.8		5.1
5	3.0		6.4
7	2.4	Washout BT	7.4
23	1.0		1.5

* Cycloheximide treatment 1–9 hr after fusion.

periods (30 or 90 sec), although many irradiated cells were then dead 1 day later.

The Role of Macrophage Partner in the Masking Reaction.—Studied to determine if this process was unique to fusion with melanoma cells. As shown in Table VIII, fusion with Ehrlich ascites tumor cells caused heterokaryon receptor activity to disappear even more rapidly. Macrophage-chick erythrocyte heterokaryons on the other hand maintained their receptor activity for 7

TABLE VII

*The Effect of Ultraviolet Irradiation before Fusion on the Ingestion of AbSRBC by Heterokaryons**

Fusion	Time after fusion	1:1 Heterokaryons		Ratio heterokaryons:macrophages
		Mean	SE	
	hr			
Control	3	10.5	1.3	1.05
	9	6.6	1.6	0.67
	21	1.8	0.62	0.20
Ultraviolet-treated melanoma cells	3	11.0	1.0	1.3
	9	9.5‡	0.71	1.3
	21	7.3§	0.74	0.82
Ultraviolet-treated macrophages	3	9.9	1.0	1.0
	9	5.0	1.0	0.65
	21	1.8	0.36	0.23

* Cells were irradiated for 10 sec at 20 cm distance.
‡ $t = 1.737$, $P < 0.05$.
§ $t = 3.6066$, $P < 0.0025$.

TABLE VIII

The Role of Fusion Partner on the Ingestion of AbSRBC by 1:1 Macrophage Heterokaryons

Type of macrophage heterokaryon	Time after fusion	Ratio heterokaryon: macrophage × 100
	hr	
Melanoma cell	20	15
Ehrlich ascites tumor cells	20	0
Primary chick fibroblast	3	83
	20	49
	45	42
	70	37
12 day chick erythrocyte	20	100
	45	80
	120	105
	168	90

days. After fusion with primary chick fibroblasts, an intermediate result was obtained and receptor activity diminished only gradually.

DISCUSSION

The experiments with trypsin and inhibitors of protein synthesis suggest that masking of the macrophage receptor is a major cause of the loss in phago-

cytic function in heterokaryons. Additional membrane changes probably accompany the fusion of two cells which differ in their membrane properties. These include the spreading out and intermixing of surface enzymes (3) and antigens (8) and, perhaps, other dynamic changes in the synthesis and turnover of membrane components. The use of inhibitors in such a system could therefore bring about complex changes and their effect should be interpreted with great caution. Our ignorance of the chemical nature of the macrophage receptor (1) introduces further uncertainty. However, the stability of the receptor during trypsinization and inhibition of protein or RNA synthesis made it a particularly useful membrane marker for the present studies.

Macrophages may carry adsorbed gamma globulin on their plasma membrane (9) and trypsin treatment, which has been reported to enhance phagocytosis, could remove such antibody from the cell surface (1). In the present studies the loss and recovery of receptor function by trypsin treatment did not require the presence of serum factors. Moreover, the membrane proteins responsible for masking could be regenerated after trypsin treatment. Masking occurred as rapidly after reversal of protein synthesis inhibition as after fusion without inhibitor treatment (3). The masking process was prevented by inactivating the melanoma nucleus with ultraviolet light or by substitution with an inert chick red cell nucleus (10). The melanoma nucleus was therefore ultimately responsible for masking the macrophage membrane receptor as well as inducing macrophage DNA synthesis (6).

The masking reaction could be because of direct steric hindrance of receptors, but more complex conformational changes in membrane structure are of course possible. The insertion of new melanoma proteins into the heterokaryon membrane could, for example, displace the macrophage receptor into crypts which are then inaccessible to the sensitized red cells. The masking proteins probably belong to the melanoma cell coat since they are susceptible to proteolytic digestion, without loss of cell viability. Mild digestion with enzymes has been used to unmask several cell surface receptors and antigens in a similar fashion. Proteases have been used to augment or reveal red cell antigens (11) as well as cell receptors which react with plant lectins like wheat germ agglutinin (12) or concanavalin A (13). Similarly, SV 40-transformed tumor cells possess surface (S) antigens which become demonstrable in untransformed cells after mild proteolytic treatment (14).

Neuraminidase did not reactivate macrophage receptor activity, although it may enhance nonspecific phagocytosis (15) and perhaps unmask "immunogenicity" in malignant cells or trophoblast (16, 17). This failure was not because of the use of inactive enzyme.

The ability to mask the macrophage receptor is not unique to melanoma cells since Ehrlich ascites tumor cells masked phagocytic function in heterokaryons even more rapidly. Ehrlich ascites tumor cells also suppress the antigens of different cells after hybridization (18, 19), perhaps by a similar

masking mechanism. Fusion between macrophages and embryonic chick fibroblasts brought about a very slow decline in receptor activity. More cells have to be tested to determine if the masking property is associated with malignancy, rate of cell growth, or cell maturity.

It is interesting to compare the present findings with those made in chick erythrocyte-mouse fibroblast heterokaryons (20). The kinetics of disappearance of chick species antigens from these heterokaryons resembles that of the macrophage receptor and ultraviolet irradiation of the chick red cell before fusion also failed to prevent its disappearance. This process also depended on cell metabolism since incubation at lower temperature delayed the loss of antigens. It is unlikely, however, that this loss was because of masking, since these antigens reappear spontaneously and accumulate progressively. Do these two heterokaryon systems differ because of the particular receptor under study or because of the cells involved? Both possibilities can be tested.

It is not known if material has to be removed from the heterokaryon surface to achieve unmasking or whether cleavage of particular peptide bonds brings about a reorganization in membrane structure similar to that postulated for the unmasking of wheat germ agglutinin receptors (12). Studies with these receptors suggest that such differences in membrane structure could play an important part in regulating cell growth (21). In addition, the masking reaction has potential significance in tumor-host relationships (22). Patients with tumors often have circulating antibodies which enhance tumor growth by masking tumor antigens (23). The macrophage heterokaryons illustrate another mechanism by which a tumor cell could alter its membrane properties in such a way that it would escape immunologic surveillance (24). Tumor cells which can diminish their antigenicitity would be expected to have considerable selective advantage in vivo and a masking phenomenon could also be invoked to explain tumor progression.

SUMMARY

Mouse peritoneal macrophages possess a specific plasma membrane receptor for antibody-coated particles. Sheep red cells coated with rabbit 7S antibody attach readily to the macrophage surface and are subsequently interiorized. The fusion of macrophage with nonphagocytic mouse melanoma cells produces heterokaryons in which the macrophage receptor is drastically altered. The receptor is present shortly after fusion and heterokaryons are actively phagocytic. The ability to bind and ingest red cells is, however, progressively lost over the next 12–24 hr and does not reappear thereafter.

Exposure of heterokaryons to trypsin (1–100 μg/ml for 30 min at 37°C) results in the reappearance of initial receptor activity and the unmasking of the surface receptor. This property is again lost upon subsequent cultivation. The masking process takes place when cells are cultivated in the absence of

IgG so that the adsorption of antibody from the medium is not responsible for this phenomenon. Inhibition of heterokaryon protein synthesis preserves phagocytic activity in a reversible fashion and prevents the masking of macrophage receptors. Inhibition of melanoma RNA synthesis before fusion is also able to block subsequent masking, but is ineffective if delayed until after fusion. Ultraviolet irradiation of the melanoma cell before fusion prevents subsequent masking, whereas similar treatment of the macrophage has no effect.

Cells differ markedly in their ability to mask the macrophage phagocytic receptor after fusion. Ehrlich ascites tumor cells mask the receptor rapidly, primary chick fibroblasts minimally, and embryonic chick erythrocytes not at all.

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