SEROLOGICALLY DEMONSTRABLE ALLOANTIGENS OF MOUSE EPIDERMAL CELLS*

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(Received for publication 6 December 1971)

The antigenic composition of cell surfaces is one of the present focal points of immunogenetics, particularly as this may bear on the operation of selective gene action in specifying surfaces of cells following contrasting pathways of differentiation (1, 2). Far more is known about thymocytes from this point of view than about any other cell type, because they are readily available as viable cells in free suspension and thus can easily be analyzed by serological methods, notably by the cytotoxicity test (3, 4).

Although the cell surface antigens of skin are of great interest, this tissue has not been studied with the cytotoxicity method because of the difficulty in obtaining satisfactory preparations of viable single epidermal cells. The one exception is the recent study of Copper and Lance (5) who reported the demonstration of H-2 on enzymatically dispersed cells, with the ^{51}Cr-release cytotoxicity test (6, 7). From the standpoint of simplicity, visual reading with trypan blue (3, 4) has advantages over the ^{51}Cr-release version of the cytotoxicity test (6, 7) especially in studies that entail the testing of cells from many individual mice, when the extra steps involved in the ^{51}Cr-release test become a serious handicap; however, the trypan blue test demands cell suspensions of superior quality. We needed to apply the cytotoxicity test with epidermal cells to study serologically a new system of alloantigens, provisionally referred to as Sk[1], which are expressed on epidermal cells but not on hemopoietic tissue, and so give rise to homograft responses directed selectively against skin (8, 9).

In this report we shall describe the details of preparation that we have found to be important in obtaining epidermal cell suspensions of the standard required for the trypan blue test. With this technique we have been able to make a serological study of the surface antigenic composition of epidermal cells using a variety of cytotoxic antisera belonging to known systems of alloantigens, including Sk.

* Supported in part by National Cancer Institute grant CA 08748 and a grant from the John A. Hartford Foundation, Inc.

‡ Recipient of fellowships from the Stiftung Volkswagenwerk and the Deutsche Forschungsgemeinschaft.

[1] *Abbreviations used in this paper:* C, complement; C3H, C3H/An; B6, C57BL/6; FBS, fetal bovine serum; MBLA, mouse specific bone marrow-derived lymphocyte antigen(s); MSLA, mouse specific lymphocyte antigen(s); MSPCA, mouse specific plasma cell antigen(s); *Sk*, genetic locus (loci) specifying skin differentiation alloantigen(s).

Materials and Methods

Mice.—These were obtained from our own colonies and included the following congenic stocks (C57BL/6 is abbreviated B6; C3H/An, C3H): on a strain A (θ-C3H, TL^+) background, A/θ-AKR and A/TL^-; on an AKR ($H\text{-}2^k$) background, AKR/$H\text{-}2^b$; on a B6 ($H\text{-}2^b$, Ly-A.2, Ly-B.2, TL^-) background, B6/$H\text{-}2^k$, B6/Ly-A.1, B6/Ly-B.1, and B6/TL^+.

Antisera.—See Table I.

TABLE I

Antisera

Immunization	Abbreviated designation of antiserum in text	References
B6* anti-BALB/c ascites sarcoma Meth A	Anti-$H\text{-}2^d$	
DBA/2 anti-C57BL ascites leukemia EL4	Anti-$H\text{-}2^b$ (i)§	10
A anti-C57BL ascites leukemia EL4	Anti-$H\text{-}2^b$ (ii)	
B6/TL^+‡ anti-A strain spontaneous leukemia ASL1	Anti-$H\text{-}2^a$	
(A/θ-AKR‡ × AKR/H-2B‡)F_1 anti-A strain spontaneous leukemia ASL1	Anti-θ-C3H	
C3Hf/Bi anti-AKR thymocytes	Anti-θ-AKR (i)§	11
(B6 × A)F_1 anti-A/θ-AKR‡ thymocytes	Anti-θ-AKR (ii)	
(BALB/c × B6)F_1 anti-B6/Ly-A.1‡ thymocytes	Anti-Ly-A.1	10, 12
C3Hf/Bi anti-CE/J thymocytes	Anti-Ly-A.2	10, 12
B6/$H\text{-}2^k$‡ anti-CE/J thymocytes	Anti-Ly-B.1	10, 12
B6/Ly-B.1‡ anti-B6 leukemia ERLD	Anti-Ly-B.2	10, 12
(CBA/T6 × SJL/J)F_1 anti-C58 thymocytes	Anti-Ly-C.1	12
C58 anti-CE/J thymocytes	Anti-Ly-C.2	12
(A/TL^-‡ × B6)F_1 anti-A strain spontaneous leukemia ASL1	Anti-TL.1,2,3	
DBA/2 anti-BALB/c myeloma MOPC-70A	Anti-PC.1	13
B6 ♀♀ anti-B6 ♂ skin (4 grafts)	Anti-H-Y	14
Lethally irradiated B6 mice restored with (B6 × A)F_1 cells; serum taken after rejection of 3 strain A skin grafts	Anti-Sk.1	8, 9
Lethally irradiated A mice restored with (B6 × A)F_1 cells, serum taken after 1 or 2 B6 strain skin grafts (see text)	Anti-Sk.2	8, 9

* B6 = C57BL/6.

‡ Congenic stock.

§ These antisera are known to contain Ly antibodies also, but the specificity named is the only relevant one in the experiments described in the text.

Preparation of Epidermal Cells for Cytotoxicity Tests.—Suspensions of single epidermal cells were prepared by enzymatic dissociation (15–17) of tail skin. Preliminary tests, including comparison of trypsin, pronase, collagenase, and hyaluronidase, led us to adopt the following procedure. A circular incision was made round the base of the washed tail, and a second incision along its length. The tail skin was peeled off in one piece and incubated at 37°C for 30–45 min in 1% trypsin (Bacto-Trypsin in Puck's saline). The time required for digestion of dermal-epidermal connections varied with the strain, sex, and age of the donor. After incu-

bation the tail skin was rinsed in Ringer's solution and spread out, dermal surface up, in a dry Petri dish. With two curved forceps, the loosened dermis was gently separated from the underlying epidermal sheet, and discarded. The exposed epithelial layer was then covered with a thin layer of medium 199 containing 10% fetal bovine serum (FBS: "IPT" = immunoprecipitin-tested free of γG; Grand Island Biological Co., Grand Island, N. Y.). With the aid of a dissecting microscope, the surface was now lightly scraped with the tip of a fine-drawn, heat-polished Pasteur pipette; the overlying medium then was pipetted off and replaced with fresh medium from a second pipette held in the other hand. This process was repeated several times.

The loosened clumps of cells collected in this way were further dispersed by incubation in 0.1% trypsin for 10–20 min at 37°C. The time required for dispersion varied, as it did for the first trypsinization. Two volumes of medium 199 containing 10% FBS were now added and the cells washed three times by slow centrifugation in the cold. Finally, any remaining clumps of cells were removed by minimal slow centrifugation, single cells remaining in the supernate. The average yield was 5×10^6 cells/tail. The viable count, according to trypan blue exclusion, was 86–98%.

A Comment on the Use of Trypsinized Cells in Cytotoxicity Tests.—Trypsin produces various functional and morphological effects on the cell surface (18–22), some of them even at concentrations as low as 5 μg/ml (19, 21). According to Poste (22), and certainly in our own experience, repeated washing and addition of serum (probably to inactivate cell-bound trypsin), are important steps in preserving cell viability after trypsinization. Epidermal cells prepared as described in this report were examined by electron microscopy by our colleague Dr. Aoki. These showed the morphology typical of epidermal cells; the adverse signs reported by Edwards and Fogh (20) in other trypsinized cell types were not apparent.

It is not possible to compare trypsinized with nontrypsinized epidermal cells in the cytotoxicity test, but it is possible to make the comparison with thymocytes and lymphocytes. We have done so, and have found that trypsinization, under conditions as similar as possible to those used for epidermal cells, did not alter the reaction of normal thymocytes and lymphocytes to H-2, θ, Ly, and TL antisera in cytotoxicity tests.

Cytotoxicity Test.—The cytotoxicity test was based on the method of Gorer and O'Gorman (3) with modifications. The following conditions were found to be optimal for performing the test with trypsinized epidermal cells, and particularly for obtaining satisfactorily low counts of dead cells in controls.

Complement (C): Rabbits were individually selected for high C activity and low toxicity for thymocytes (about one rabbit in five is acceptable). The selected serum was absorbed with a pool of various mouse cells, including epidermal cells, to remove heteroantibody, the absorption being carried out in the presence of ethylenediaminetetraacetic acid (EDTA) to bind divalent cations and so prevent loss of C during absorption. (For a full description of the method, which permits the use of relatively high concentrations of C without prohibitive toxicity, see reference 23.)

Medium: In a preliminary survey, several media were tested for use in the preparation of epidermal cells and as diluents of antiserum and C in the cytotoxicity test. Medium 199 was chosen; the addition of 10% FBS was found to aid considerably in maintaining viability and did not noticeably diminish the sensitivity of the cytotoxicity test.

Procedure: Equal volumes (0.05 ml) of (a) antiserum, serially diluted, (*b*) pooled absorbed rabbit serum (C source, diluted 1:8), and (*c*) cells (5×10^6/ml) were incubated for 45 min at 37°C. Cell counts, live and dead, were then made after addition of 0.1 ml freshly prepared 0.16% trypan blue. Controls were always included in which the test cells were incubated with (*a*) antiserum alone, and (*b*) C alone. They contained less than 20% stained (dead) cells; these control data are not included in the tables.

Typing of Cells or Tissues for the Presence or Absence of Cell Surface Antigens by the Method of Absorption.—(For details see reference 24.) Antiserum of the required specificity was ti-

trated (in doubling dilutions) against the relevant test cell, and a dilution two or three points below the end point selected. An aliquot of this diluted serum was mixed with an equal volume of washed, packed, viable cells, or washed tissue homogenate, and incubated in test tubes of 0.8 ml capacity for 30 min on a shaker in the cold room. The absorbed serum was recovered by centrifugation in the cold and tested against the relevant cells for residual cytotoxic activity. Brain tissue was prepared for absorption tests by mincing with scissors, followed by gentle homogenization in a Teflon tissue homogenizer (Tri-R Instruments, Inc., Rockville Centre, N. Y.) in Earle's balanced salt solution. The homogenate was then washed three times.

RESULTS

I. *H-2 Antigen.*—H-2 antisera are cytotoxic for epidermal cells (as first shown by Cooper and Lance [5]). The specificity of these reactions was con-

TABLE II

*Demonstration of H-2 Antigen on Epidermal Cells**

Cell donor		Cytotoxicity test on epidermal cells: $H\text{-}2^b$ anti-$H\text{-}2^a$ serum dilution (% cells dead) 1/8	1/16	Results (+ or −)	Hemagglutination test (reference 25) $H\text{-}2^b$ anti-$H\text{-}2^a$ serum results (+ or −)
Controls	A ($H\text{-}2^a$)	66	72	+	+
	(B6 × A)F_1	58	60	+	+
	B6 ($H\text{-}2^b$)	16	16	−	−
Backcross mice	1	53	44	+	+
	2	20	17	−	−
	3	50	48	+	+
	4	53	50	+	+
	5	15	17	−	−
	6	45	44	+	+

* Specificity is confirmed by these cytotoxicity tests on epidermal cells of (B6 × A)F_1 × B6 backcross mice independently typed for H-2 by hemagglutination.

Other similar tests have invariably shown concordance between the two tests.

firmed (*a*) by the use of H-2 antisera prepared in H-2–congenic stocks, (*b*) by positive tests with epidermal cells taken from congenic mice differing from the donors of the antiserum only at the *H-2* locus, (*c*) by negative tests with H-2 antisera used in conjunction with epidermal cells of the antiserum-producing strain, and (*d*) by cytotoxicity tests on epidermal cells from backcross mice typed independently by hemagglutination (an example is given in Table II). H-2 cytotoxic titers were considerably lower with epidermal cells than with lymphocytes (see also reference 5). Reactions were also markedly weaker with heterozygote-positive than with homozygote-positive, which is characteristic of relatively insensitive systems.

II. *θ Antigen.*—

(*a*) *Reaction of epidermal cells with θ antisera in cytotoxicity tests:* All *θ* antisera tested were cytotoxic for epidermal cells of mice carrying the pertinent *θ* allele, but not for epidermal cells of mice carrying the alternative allele. One

of these θ antisera was prepared in congenic mice differing only for θ type. A confirmatory test is shown in Table III. Eight A strain mice and seven A/θ-AKR congenic mice were amalgamated into a single group; epidermal cells from each of them were then typed with θ antisera while a second investigator typed their thymocytes in the usual way. A comparison of the results showed complete concordance between the two tests.

TABLE III

*Demonstration of θ Antigen on Epidermal Cells**

Cell donor		Cytotoxicity test on epidermal cells				Typing of thymocytes	
		Antisera (dilution 1/8)					
Strain	Mouse number	Anti-θ-C3H	Anti-θ-AKR		Result	Anti-θ-C3H	Anti-θ-AKR (ii)‡
			(i)‡	(ii)‡			
A(θ-C3H)	1	80	20	12		+§	−‖
	2	70	20	12		+	−
	3	68	19	12		+	−
	4	66	20	18	θ-C3H	+	−
	5	70	22	18		+	−
	6	76	19	15		+	−
	7	76	13	13		+	−
	8	82	11			+	−
A/θ-AKR	1	14	72	60		−	+
(congenic)	2	13	61	50		−	+
	3	18	70	50	θ-AKR	−	+
	4	15	78	60		−	+
	5	13	78	65		−	+
	6	14	77			−	+
	7	19	80			−	+

* Specificity confirmed by independent typing of epidermal cells and thymocytes of A strain (θ-C3H) and congenic A/θ-AKR mice; "blind" tests on a single amalgamated group of 15 mice.

‡ Different antisera: see Table I.

§ + = > 90% cells dead.

‖ − = < 15% cells dead.

Specificity was further confirmed by absorption. As Table IV indicates, epidermal cells of θ-C3H type removed all activity of anti-θ-C3H sera against θ-C3H thymocytes and epidermal cells and this absorption is serologically specific: θ-AKR epidermal cells did not absorb activity from the same serum.

(*b*) *Production of θ antibody by A mice grafted with skin from congenic A/θ-AKR mice:* As θ antigen was seen to be present on epidermal cells, it seemed likely that skin grafts between θ-incompatible mice would induce the formation of θ antibody. This was confirmed by grafting A ♀ mice with congenic A/θ-AKR skin; Fig. 1 shows that the grafted mice formed θ antibody, the specificity

TABLE IV

Presence of θ Antigen on Epidermal Cells Confirmed by Their Capacity to Absorb θ Antibody

Anti-θ-C3H serum (1/8) absorbed with*	Cytotoxicity test with absorbed antiserum (diluted 1/2) on A strain cells		Result of absorption
	Thymocytes (% dead)	Epidermal cells (% dead)	
A strain (θ-C3H)			
Thymocytes	20	19	+
Epidermal cells	19	17	+
A/θ-AKR (θ-AKR)			
Thymocytes	>95	55	−
Epidermal cells	>95	60	−
Control: no absorption	>95	55–60	

* Method: 0.2 ml anti-θ-C3H serum (diluted 1/8) incubation with 0.2 ml of packed, washed cells for 30 min at 4°C.

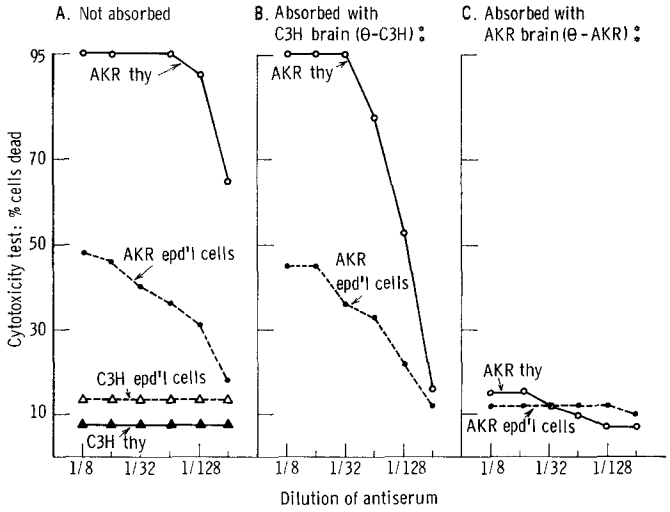

FIG. 1. Demonstration of θ antibody in the serum of A (θ-C3H) mice grafted with skin from congenic A/θ-AKR mice. (A) The serum is cytotoxic for AKR strain epidermal cells and thymocytes, but not for C3H (θ-C3H) epidermal cells or thymocytes. (B) Absorption with AKR brain does not remove activity for thymocytes or epidermal cells, whereas (C) absorption with C3H brain removes activity for both thymocytes and epidermal cells. ** Method: 0.2 ml serum (diluted 1/8) incubated with 0.2 ml of packed washed hrain homogenate for 30 min at 4°C.

of which was confirmed by positive and negative absorption tests with AKR and C3H brain, respectively, as indicated in the figure. The cytotoxic reaction against lymph node lymphocytes (not shown in the figure) was considerably weaker than against thymocytes, which is characteristic of the θ system. θ

antibody was found in the serum of several mice whose grafts showed no signs of rejection (see Discussion).

III. *Sk Antigen.*—Although the Sk system of antigens may eventually prove to comprise more than one *Sk* genetic locus, it will be referred to here as if it were governed by alternative alleles at a single *Sk* locus, specifying Sk.1 antigen in the A strain and Sk.2 antigen in B6.

(*a*) *Serology of Sk.1 antigen:* Lethally irradiated B6 mice restored with (B6 × A)F_1 hemopoietic cells reject (B6 × A)F_1 and A strain skin although they remain chimeric indefinitely (8). The serum anti-Sk.1 was obtained from such chimeras that had rejected three A strain skin grafts (see Table I).

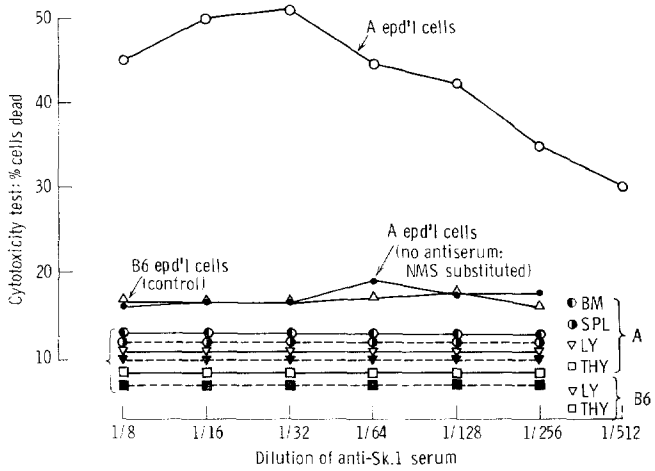

FIG. 2. Cytotoxicity tests on epidermal cells, and other cells, with anti-Sk.1 serum [from (B6 × A)F_1 → B6 radiation chimeras that rejected (B6 × A)F_1 skin grafts]. The cytotoxic reaction is specific for A strain epidermal cells; B6 epidermal cells are not lysed, nor are bone marrow cells, spleen cells, lymph node lymphocytes, or thymocytes of either B6 or A strain origin. NMS = normal mouse serum.

Anti-Sk.1 serum was cytotoxic for A strain epidermal cells but not for the B6 epidermal cells, nor for cells of thymus, lymph nodes, spleen, or bone marrow from either strain of mouse (Fig. 2). These serological findings accord with previous interpretations of skin homograft reactions directed to differentiation alloantigens present on skin and absent from hemopoietic tissue (Sk alloantigens).

Absorption analysis of anti-Sk.1 serum confirmed the absence of Sk.1 antigen on thymocytes, lymph node lymphocytes, and spleen cells, and revealed that it is present on brain (Table V). The specificity of absorption by brain is confirmed in Table V by the failure of Sk.1$^-$ brain (B6) to absorb anti-Sk.1 antibody.

The presence of Sk.1 antigen on brain was further confirmed by the finding

that the transplanted A strain neuroblastoma C1300 is sensitive to anti-Sk.1 serum (but not to anti-Sk.2 serum [see below]), to about the same degree as A strain epidermal cells (Fig. 3). A number of other tumors were tested with anti-Sk.1 serum, but none was found sensitive (Table VI).

TABLE V

Representation of Sk.1 Antigen on Cells of Various Tissues Determined by Their Capacity to Absorb Sk.1 Antibody

Anti-Sk.1 serum (1/8) absorbed with*	Cytotoxicity test with absorbed antiserum (% A epidermal cells dead)		Result of absorption
	1/1‡	1/2‡	
A strain (Sk.1$^+$)			
Epidermal cells	15	15	+
Brain	14	14	+
Thymocytes	40	42	−
Lymph node lymphocytes	42	43	−
Spleen cells	39	42	−
B6 (Sk.1$^-$) (controls)			
Epidermal cells	40	42	−§
Brain	40	49	−§

* For method see footnote to Table IV.

‡ Residual cytotoxic activity of absorbed antiserum against A strain epidermal cells without further dilution (1/1) or diluted (1/2).

§ These negative results show that absorption of anti-Sk.1 antibody by A strain epidermal cells and brain (above) was serologically specific.

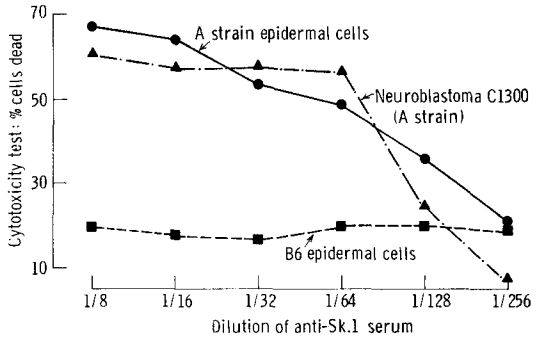

FIG. 3. Sk.1 antigen is present on neuroblastoma C1300 cells of the A strain.

(*b*) *Serology of Sk.2 antigen:* Similar chimeras in which the other component of the cross is used as the recipient, i.e. irradiated A mice restored with (B6 × A)F_1 hemopoietic cells, formed the antibody anti-Sk.2 when grafted with B6 skin. Not all such grafts are rejected (8), but we find that those chimeras that retain their B6 grafts without evidence of rejection frequently have good titers of anti-Sk.2 antibody, not significantly lower than those

chimeras that rejected their grafts. This retention of skin grafts despite the formation of antibody to epidermal cell antigens is similar to what was observed in the θ system (see section II above).

(*c*) *Linkage:* The only loci for which close linkage has so far been excluded are *H-2* and *Tla*, indicated by typing epidermal cells of congenic mice with anti-Sk.1 and anti-Sk.2 sera (Table VII).

TABLE VI

Cytotoxicity Test for Sk.1 Antigen on Various Tumors

Tumor*	Mouse strain of origin	Ascites or subcutaneous transplant	% cells lysed by anti-Sk.1. serum (1/8)	Result
Spindle-cell sarcoma Sa1 (*a*)	A	Ascites	5	–
Epithelioma DMBA 788 (*b*)	XVII	Subcutaneous‡	10	–§
Epithelioma DMBA 592 (*b*)	XVII	Subcutaneous‡	10	–§
Anaplastic carcinoma 15091A (*a*)	A	Subcutaneous‡	10	–
Adenocarcinoma TA3/Ha41 (*c*)	A/HeHa	Ascites	5	–
Spontaneous mammary carcinoma (*d*)	A/θ-AKR	Subcutaneous‡	15	–
Neuroblastoma C1300 (*a*)	A	Subcutaneous‡	60	+

* Tumors obtained from: (*a*) N. Kaliss, The Jackson Laboratory, Bar Harbor, Maine; (*b*) G. Pasternak, Deutsche Akademie der Wissenschaften, Institut für Medizin und Biologie, Berlin-Buch; (*c*) T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, N.Y.; and (*d*) our own stock.

‡ Cells prepared for cytotoxicity test by trypsinization.

§ As the *Sk* genotype of strain XVII is not known, the negative results do not necessarily signify that these epitheliomas lack Sk alloantigens.

TABLE VII

Close Linkage of Sk with H-2 or Tla is Excluded

Typing of epidermal cells	Strains
Sk.1$^-$/Sk.2$^+$	B6, B6/TL$^+$, B10.A (C57BL background)
Sk.1$^+$/Sk.2$^-$	A, A/TL$^-$, A.BY (A background) also AKR, C3H, CBA(Lyon)

IV. *H-Y (Male) Antigen.*—Anti–H-Y serum was obtained from B6 females that had rejected three or more B6 male skin grafts. This antiserum is cytotoxic for sperm, and the reaction is specific for H-Y antigen (14). Anti–H-Y serum is not cytotoxic for cells from thymus, spleen, lymph nodes, or bone marrow, although these tissues absorb anti–H-Y activity; evidently H-Y antigen is present in amounts too small to give a positive test, or it is present on only a small proportion of such cells (14); the former seems more probable.

Anti–H-Y serum was strongly cytotoxic for male epidermal cells (Fig. 4,

Table VIII). Specificity was confirmed by showing that male epidermal cells, but not female epidermal cells, removed all cytotoxic activity against male epidermal cells (Table VIII).

As noted previously with sperm (14) there were substantial differences among mouse strains in the sensitivity of male epidermal cells to anti-H-Y serum (Fig. 4). Further work is needed to establish whether these differences are due to (*a*) strain differences in susceptibility to complement-mediated cytolysis (as we have observed in another system), (*b*) strain differences in the amount

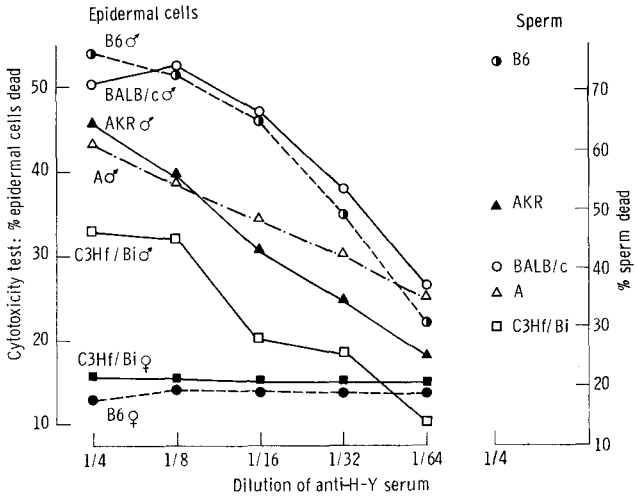

FIG. 4. Cytotoxic effect of anti-H-Y serum (from B6 female rejectors of B6 male skin) on male epidermal cells of various strains. Anti-H-Y serum was positive against male epidermal cells of all strains tested with marked differences in sensitivity from strain to strain, but not against female epidermal cells. On the right are shown the percentages of sperm lysed by anti-H-Y serum, the sperm donors being selected from the strains whose epidermal cells were tested (left). It is seen that the sensitivity of sperm tends to parallel the sensitivity of epidermal cells for each strain.

of H-Y antigen present on the cell surface, or (*c*) strain differences in the antigenic specificities of H-Y antigen (see reference 26).

V. *TL, Ly-A, Ly-B,C, and PC Alloantigens.*—The antisera which belong to these systems and which we have tested against epidermal cells are listed in Table I; none gave a positive cytotoxicity test. One antiserum, anti-Ly-B.1, was also tested by absorption with epidermal cells of Ly-B.1 mice, which failed to remove cytotoxic activity against Ly-B.1 thymocytes. We conclude that Ly antigens are lacking on epidermal cells. Similarly anti-PC.1 and anti-TL.1, 2, 3 sera were not cytotoxic for epidermal cells, nor did epidermal cells from PC.1 or TL.1, 2, 3 mice remove anti-PC.1 or TL.1, 2, 3 activity in the standard absorption test for PC.1 or TL.1, 2, 3.

DISCUSSION

Genetic Specification of Cell Surface Composition of Differentiated Cells.—The serological investigation of cell surfaces has usually centered on antigenic differences among individuals of a species, a thorough comprehension of which is the essence of transplantation biology. The serological techniques used for that purpose can be applied equally to studying surface antigenic differences among various cell populations in a single individual. A priori, such phenotypic differences are likely to have wider biological relevance than the genotypic variation responsible for histoincompatibility, because they imply that there are patterns of selective gene action which govern the composition of different cell surfaces in accord with the requirements of particular pathways of differentiation; and this may well be critical to the problem of relating genetics to

TABLE VIII

Specificity of the Anti-H-Y Cytotoxic Reaction on Male Epidermal Cells Confirmed by Absorption Tests with Male or Female Epidermal Cells

Anti-H-Y serum (1/8) absorbed with*	Cytotoxicity test with absorbed antiserum (% B6♂ epidermal cells dead)		Result of absorption
	1/1‡	1/2‡	
B6♀ epidermal cells	57	60	−
B6♂ epidermal cells	15	16	+
Control: no absorption	55	57	

* For method see footnote to Table IV.

‡ Residual cytotoxicity of absorbed serum tested against B6 epidermal cells without further dilution (1/1) or diluted (1/2).

morphogenesis and to other manifestations of cellular interaction, as discussed more fully elsewhere (2).

The data summarized in Table IX, which is an inventory of surface antigens classified according to the types of cell on which they appear, can be viewed as a fragment of the mouse's genetic program of cell surface specification, for the presence of a certain set of components on one cell, and a different set on another, is attributable to selective gene action, regardless of whether the presence or absence of antigen at accessible sites is directly due to expression vs. nonexpression of structural genes coding for these antigens, or to a less direct genetic mechanism.

The total surface of a cell could in theory be described in terms of an assembly of components specified by a certain set of genes (2). What proportion of these genes are expressed only conditionally, i.e., only in consequence of a particular mode of differentiation? A hint of the answer comes from the fact that of the many systems of antigens demonstrable serologically on nucleated cells of the mouse, only H-2 is expressed on apparently all cells (1), suggesting that the

proportion of conditionally expressed loci may indeed be high. So it may be that differentiation is the source of more radical differences in cell surface composition than is intraspecies genetic variation, or even than the wider genetic divergences separating species, genera, etc. This is somewhat contrary to past impressions and the case for it is argued from other aspects and in more detail elsewhere (2).

Other Considerations.—The antigenic phenotype of epidermal cells revealed by this study has several implications, especially for transplantation biology:

(*a*) It is important that the Sk alloantigens are now identified serologically as differentiation alloantigens confined to epidermal cells and brain, because their demonstration has previously depended wholly on the results of grafting in vivo (8, 9). Recognition of the Sk system removes much of the mystery from what formerly seemed to be anomalous cases of skin rejection by hemopoietic

TABLE IX

Selective Gene Action Exemplified for Several Loci Coding for Cell Surface Components

	H-2	H-Y	Sk	θ	Pca	Ly-A	Ly-B,C	Tla
Epidermal cells	+	+	+	+	−	−	−	−
Sperm	+	+	−	−	−	−	−	−
Brain	+	+	+	+	+	−	−	−
Plasma cells	+		−	−	+	−	−	−
Lymphocytes	+	+	−	+	−	+	+	−
Thymocytes	+	+	−	+	−	+	+	+
Erythrocytes	+		−	−	−	−	−	−

+ = expressed.
− = not expressed.

chimeras, and from at least some instances of "split tolerance." To take an example of the latter, the rejection of C57 skin grafts by [(CBA × C57) → A] chimeras, in the face of acceptance of CBA skin grafts (27, 28), is readily understood on the basis of our Sk typing of epidermal cells from the three strains concerned, for A and CBA epidermal cells type as Sk.1, and C57 (B6) types as Sk.2 (Table VII).

Presumably the observed qualitative differences in antigen phenotype among contrasting cell populations in the same individual, arising evidently from selective gene action geared to cellular differentiation, contribute to observed differences in the fate of grafts of different organs or tissues from donors of the same genotype (29–35), and to the different tolerance-inducing capabilities of contrasting cell populations (cf. especially references 34–39). The relevance of this to tissue typing for purposes of human organ transplantation is self-evident.

(*b*) There has been considerable discussion of the possible role of "passenger" leukocytes in immunizing the recipients of tissue grafts, and so facilitating rejection (40–42). The fact that rejection of Sk-incompatible skin grafts by

chimeras may occur rapidly and is attributable solely to Sk incompatibility, meaning by definition incompatibility due to antigens not carried by leukocytes, indicates that the presence of leukocytes sharing pertinent antigens with epidermal cells is not essential to a rapid and fully effective skin homograft response (whatever the part played by passenger leukocytes may be under other conditions of grafting). And, conversely, isolated θ incompatibility is often or even usually insufficient to cause rejection of skin grafts despite the fact that θ is carried by passenger lymphocytes, as well as by epidermal cells.

(*c*) A point of possible clinical relevance concerns the reputed immunological basis of such skin disorders as pemphigus and bullous pemphigoid, in which serological evidence of anti-skin immune responses has been obtained (43–45). Realization that the surface antigen phenotype of epidermal cells is characteristically distinguished from that of immunocompetent cells of the same individual mouse suggests that the source of autoantigens involved in such human diseases may be the general class of cell surface antigens that are expressed on epidermal cells but not on immunocompetent cells. Experience with lymphoid cell differentiation antigens (1) indicates that the class of "skin differentiation antigens" will prove to include antigens shared by all members of the species (comparable with mouse specific lymphocyte antigen[s] [MSLA, 46], mouse specific bone marrow–derived lymphocyte antigen[s] [MBLA, 47], and mouse specific plamsa cell antigen[s] [MSPCA, 48] on lymphoid cells) as well as skin differentiation alloantigens of the type represented by Sk (although this has not so far been investigated with the necessary heteroantisera). The former are perhaps more likely candidates as autoantigens. (See references 49–52 for relevant studies with immunofluorescence.) In general perhaps the spectrum of tissues affected in different autoimmune diseases reflects the differential expression ("tissue representation") of the particular cell surface differentiation antigen(s) involved in each case. This is not to say anything very new about autoimmunity, and is intended only to stress (*a*) the importance of antigens at the cell surface as the target of immunological attack leading to tissue injury, and (*b*) that differential surface antigenicity has now been proved for nonlymphoid cells (epidermal cells) which do not occupy a "privileged" (53, 54) site.

(*d*) The serological demonstration of Sk alloantigens is invaluable for purposes of future genetic analysis, and in proving the existence of skin-selective homograft rejections, a possibility that had been entertained for some years (see e.g. references 31, 34, 35, 55) but has only recently been substantiated by critical experiments (8, 9). There will doubtless be discussion of the biological role of Sk antibodies, as indeed there continues to be discussion of the role of humoral antibodies, as opposed to cell-mediated immunity, in graft rejection generally. Certainly the presence of Sk antibodies (and as we find in preliminary grafting experiments in the θ system, the presence of θ antibodies) does not signify that rejection has taken place or will take place. We commonly find that

some [(B6 × A) → A] chimeras retain B6 skin grafts apparently indefinitely despite the formation of anti-Sk.2 antibody, and that the same is often true of accepted θ-incompatible skin grafts (see Results II *b*). This suggests comparison with the description given by Hellström of other situations where incompatible tissue is retained despite antibody production (56–60). As Hellström et al. (56–60) and Voisin et al. (61) point out, tolerance does not necessarily involve total abrogation of the immune response, and in some instances immunological enhancement may be the most appropriate mode (l62, 63), but clearly the situation with tolerated hemopoietic allografts (hemopoietic chimerism) is not to be equated with situations in which Sk-incompatible skin allografts are tolerated, as described in this report. In other words, although on the one hand the Sk cytotoxic antibody response of those radiation chimeras that happen to retain Sk-incompatible skin grafts may be no different from that of apparently identical radiation chimeras that happen to reject them, on the other hand acceptance of hemopoietic grafts, necessary for the establishment of those radiation chimeras, is never (as far as we can tell) accomplished in the face of an anti-H-2 or any other antibody response against hemopoietic cells that is recognizable by conventional cytotoxicity or hemagglutination tests.

(*e*) Of all the antigen systems now identified on epidermal cell surfaces, the one that most demands further definition is Sk, because of its relevance to so many facets of cell surface specification and of transplantation biology. To begin with, Sk.1 and Sk.2 may in fact represent more than one genetic system, and the particular vigor of A strain graft rejection by [(B6 × A) → B6] chimeras may reflect a summation of multiple Sk incompatibilities rather than one major Sk incompatibility; (we recall that the first Ly antisera to be analyzed turned out to identify two genetically separate Ly systems [10]). Genetic tests will be necessary to decide this. Identification of a linkage group or groups is important in this respect; all we know so far is that there is no close linkage of *Sk* with *H-2*:*Tla*. A major question is, "Why are Sk incompatibilities usually not associated with such vigorous rejection as that seen in [(B6 × A) → B6] radiation chimeras?" There are several well-known possibilities that have been thoroughly aired in connection with other systems in which graft rejection is inconsistent, but one is perhaps of special interest, and that is the explanation raised by the finding that mice with accepted Sk-incompatibile or θ-incompatible skin grafts may nonetheless be producing Sk or θ antibody. This cannot fail to suggest that the answer may lie in the quality of the immune response, as reflected in the balance between the cell-mediated immune response and the production of humoral antibody, which in weak systems particularly may decide whether the outcome will be acceptance or rejection.

SUMMARY

Single cells were prepared from mouse tail epidermis by a method which gives high viability counts and so permits their use in cytotoxicity tests.

According to tests with standard alloantisera, the antigen phenotype of mouse epidermal cells is H-2$^+\theta^+$Sk$^+$H-Y$^+$TL$^-$ Ly-A$^-$ Ly-B,C$^-$ PC$^-$.

The skin differentiation alloantigen Sk, which is responsible for homograft reactions directed selectively against skin, is expressed also on brain, but not on other cell types; it is present on the transplanted neuroblastoma C1300. Cytotoxicity tests with epidermal cells of H-2 congenic mouse stocks confirm that the *Sk* locus is not closely linked to *H-2*.

The lymphoid cell differentiation antigen θ also is present on both epidermal cells and brain. Mice frequently retain θ-incompatible or Sk-incompatible skin grafts although they have formed substantial titers of θ or Sk antibody in response to grafting.

Male (H-Y) antigen is demonstrable on epidermal cells by cytotoxicity tests with H-Y antibody, as it is also on one other type of cell, spermatozoa.

We are indebted to Miss Chika Iritani for the H-2 hemagglutination tests, to Mrs. Ellen Goldberg for the cytotoxicity tests on sperm, and to Miss Mary John for assistance in preparing antisera and complement.

Note Added in Proof.—Hildemann and Pinkerton, using a hemolytic plaque assay, report the presence of H-Y antigen on erythrocytes of male mice (Hildemann, W. H., and W. Pinkerton. 1966. Alloantibody production measured by plaque assay in relation to strong and weak histoincompatibility. *J. Exp. Med.* **124:**885).

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