ASSOCIATION OF THE H-Y MALE ANTIGEN WITH β_2-MICROGLOBULIN ON HUMAN LYMPHOID AND DIFFERENTIATED MOUSE TERATOCARCINOMA CELL LINES*

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The male specific antigen (H-Y) has been implicated in the rejection of male skin grafts by syngeneic female mice and with the production of anti-H-Y antibodies (1–4).

Antisera against the mouse H-Y antigen react with heterogametic cells in mammals (including man), birds, and amphibians (5–8). Thus, the H-Y antigen seems to be phylogenetically conserved, and it has been argued that the serologically detectable H-Y antigen has an invariant function in testis induction during embryonic development (9).

It has also been suggested (10) that the H-Y antigen is associated with antigens of the major histocompatibility complex (MHC)[1] on the plasma membrane, the MHC antigens β_2-microglobulin complex serving as the anchorage site for the H-Y antigen. By using rat anti-H-Y antisera, we have studied the association of the H-Y antigen with the complex formed by the major histocompatibility antigens and β_2-microglobulin on human lymphoid cells and mouse teratocarcinoma cells.

The results reported here demonstrate that the H-Y antigen is associated with β_2-microglobulin on the cell membrane.

Materials and Methods

Immune Sera

ANTI-H-Y ANTISERA. Anti-H-Y antisera were prepared in 8- to 12-wk-old female inbred Lewis rats by intraperitoneal injection of 2×10^6 or 4×10^7 spleen cells from male inbred Lewis rats. After six to eight weekly injections, rats were bled and the sera were stored at −28°C. Before use, the antisera were heat inactivated and absorbed with an equal volume of packed human female AB erythrocytes for 1 h at 4°C. Normal female Lewis rat serum was used as control.

ANTI-β_2-MICROGLOBULIN. Rabbit antimurine β_2-microglobulin serum (no. 7036) was kindly

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[1] *Abbreviations used in this paper:* EC, embryonal carcinoma; MHC, major histocompatibility complex; PBS, phosphate-buffered saline.

supplied by Dr. N. Tanigaki (11, 12). Two different rabbit anti-human β_2-microglobulin sera were obtained from Doctors A. Colle and N. Tanigaki, respectively (13).

ANTI-HLA SERA. A rabbit anti-HLA serum was kindly provided by Dr. N. Tanigaki. This xenogeneic antiserum was absorbed twice with an equal volume of Daudi cells, a human lymphoblastoid cell line lacking HLA antigen (14–16). Human anti-HLA sera were obtained from multiparous women. Only eluates (17) of anti-HLA sera from platelets were used. We had previously ascertained that neither the rabbit anti-β_2-microglobulin nor the anti-HLA used was contaminated by anti-H-Y activity (the anti-β_2-microglobulin or anti-HLA sera absorbed on female cell lines no longer react with Raji cells).

Cell Lines

HUMAN CELL LINES. The human lymphoblastoid cell lines were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. They were obtained from Doctors J. Littlefield, G. Klein, W. Bodmer, P. Goodfellow, and P. Wernet and from our own laboratory.

The cell lines were derived either from Burkitt lymphomas: Daudi (14), Raji (18), Chevalier (unpublished data), Ramos (19), BJAB (20), Namalwa (21), or from leukemic patients: SKL-1 (22), RPMI 8866. The other lymphoid cell lines were derived from normal peripheral lymphocytes (23, 24).

SOMATIC HYBRID CELLS. The origin and karyotypic characterization of the human 8 A Raji × Daudi hybrid have been described previously (25). The human hybrid Daudi × D 98/AH-2 was obtained by the Sendai virus fusion technique (26). D 98/AH-2, a line derived from HeLa female cells, is deficient in hypoxanthine phosphoribosyl transferase (27). The hybrid Daudi × D 98/AH-2 clone was selected in hypoxanthine, aminopterin, thymidine selective medium (28).

The human Y chromosomes have been identified on all human cell lines by the "atebrin fluorescence technique" (29) and a C banding technique (30). A minimum of 10 metaphases were analyzed for each cell line.

Mouse Teratocarcinoma. A number of different embryonal carcinoma cell lines (EC) and their differentiated derivatives were used. The culture and differentiation of embryonal carcinoma cells have been described previously (31). After 10–14 or 20 days in culture, differentiated cells were replated 48 h before testing. The cells were detached by treatment with EDTA solution (2 mM in phosphate-buffered saline [PBS]) and replated at 1/3 the initial density.

Two of the cell lines were derived from testicular teratocarcinoma OTT 6050 of mouse strain 129/Sv: the nullipotential F9 line (32) and the multipotential PCC3/A/1 line (31). In neither of these lines could a Y chromosome be detected by cytogenetic techniques (31, 33, 34).

The multipotential EC line PCC7-S and its azaguanine-resistant clone PCC7-S-Aza$^{R}_{1}$ were isolated from a spontaneous testicular teratocarcinoma arising in a recombinant (129 × B6) inbred line (F7). PCC7-S is a typical EC line in respect of morphological (i.e. low cytoplasm/nucleus ratio) and immunological characteristics (presence of F9 antigen according to the method previously described) (32). It gives tumors that contain well-differentiated tissues belonging to the three embryonal germ layers. Differentiation in vitro gives preferentially nervous derivative types (S. Pfeiffer, unpublished results).

The cytogenetic analysis of PCC7-S as well as of its azaguanine-resistant clone is apparently euploid: 40 chromosomes with X and Y chromosomes easily identifiable (Fig. 1). The Y chromosome can be shown to be present in tissues of the tumors even after continuous exponential growth for more than 2 mo. No preferential Y chromosome loss was observed under our in vitro culture conditions.

Sperm cells were prepared following the technique described previously (13, 35).

Serological Techniques. A microlymphocytotoxicity test was used according to Mittal (36). Selected rabbit complement was diluted 1:2 with fresh human AB serum before use. Raji cells were used as target cells in the standard assay when quantitative absorptions were performed as follows: 20 μl of undiluted antiserum was mixed with variable amounts of cells for 1 h at 4°C. After centrifugation, the supernate was tested for cytotoxic activity.

The indirect immunofluorescence technique was used on human and mouse cells in culture. Goat anti-rat IgG (Hyland Diagnostic Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) labeled with fluorescein or rhodamine were adsorbed (vol/vol) at 4°C by human or mouse fibroblasts.

Aliquots of the absorbed antiglobulin were stored at −40°C. Sequential binding of anti-H-Y

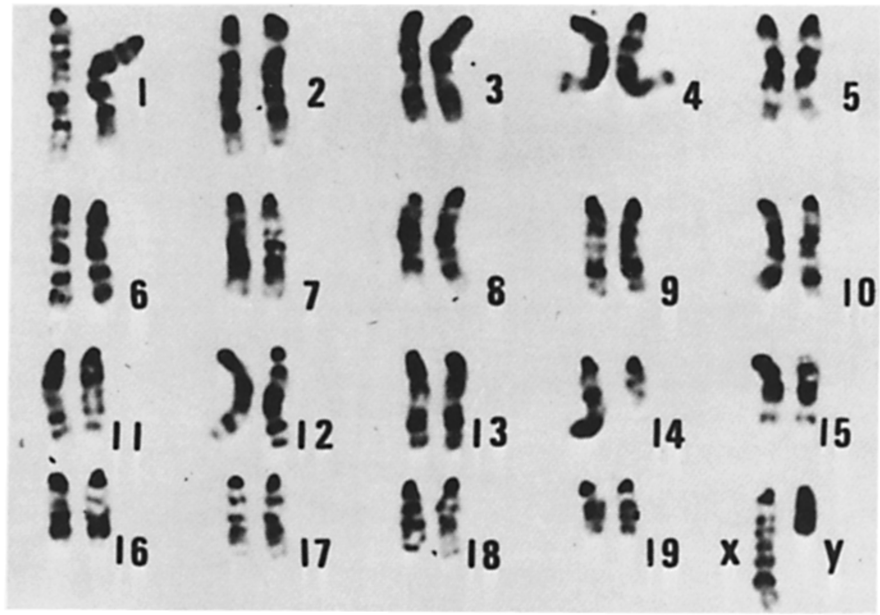

FIG. 1. Banding pattern of a PCC7-S cell. Heat denaturation followed by trypsin treatment. The abnormality of one of the chromosomes 14 is not regularly observed. The Y chromosome is present in all metaphases.

and antiglobulin sera was performed as follows: 100 μl of anti-H-Y at various dilutions was added to a pellet of 5×10^6 cells. After incubation for 1 h at 4°C with occasional shaking, the suspension was diluted with PBS and 4% bovine serum albumin and washed twice. The pellet was resuspended in 100 μl of antiglobulin at a dilution of 1:30, incubated for 40 min at 4°C, and washed three times. The final pellet was spread on a microscope slide, air dried, and fixed in the cold with methanol. The smear was then mounted in 80% glycerol in PBS and observed with both an epi-illumination fluorescence and a phase contrast microscope.

For HLA and β_2-microglobulin labeling we followed the same procedure; goat anti-human IgG and goat anti-rabbit IgG labeled with fluorescein (Hyland Laboratories) or rhodamine were absorbed on human or mouse fibroblasts and stored at −40°C before used. The relationship between H-Y, β_2-microglobulin and HLA antigen has been studied by the differential redistribution immunofluorescence method (37).

Results

Presence of the H-Y Antigen on Raji Cells. Raji is a male lymphoid cell line established from a Burkitt lymphoma (18). The results of cytotoxicity tests performed on Raji cells using a rat anti-H-Y serum (properly absorbed, see Materials and Methods) are given in Fig. 2. 90–100% of Raji cells were killed by anti-H-Y serum at 1/1 to 1/8 dilution. This cytotoxic activity was completely abolished after absorption by 10 different male lymphoid lines or by absorption with peripheral lymphocytes from 5 different male donors. The cytotoxic activity was not changed after absorption with female lymphoid cell lines or peripheral lymphocytes from female donors.

Because the H-Y antigen has previously been found on sperm (5–8) of several mammalian species including man but not on chicken spermatozoa, absorption of the anti-H-Y activity by sperm cells was studied (Fig. 3). The cytotoxic

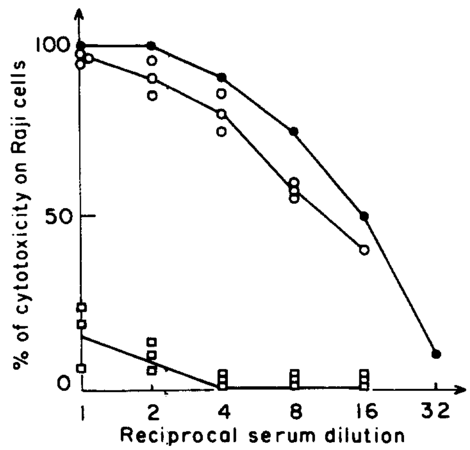

FIG. 2. Cytotoxic activity of anti-H-Y sera on Raji cells. Curve (●), unabsorbed serum; curve (□), serum absorbed with three different human male lymphoid lines; curve (○), serum absorbed with three different human female lymphoid lines. For absorption, see Materials and Methods.

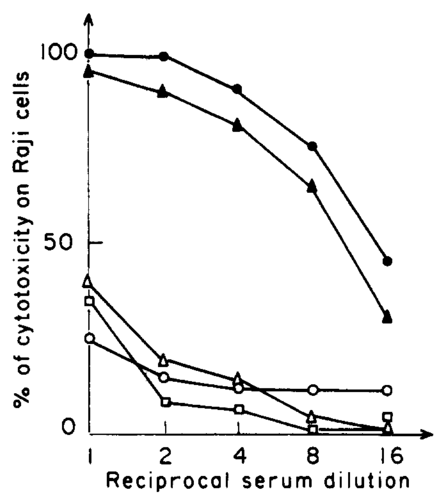

FIG. 3. Cytotoxic activity of anti-H-Y serum on Raji cells. Curve (●), unabsorbed serum. The anti-H-Y serum is absorbed with human sperm (△); mouse spermatozoa (○); pig spermatozoa (□); chicken spermatozoa (▲). For absorption, see Materials and Methods.

activity of the rat anti-H-Y sera on Raji cells was removed after absorption with mouse, pig, and human spermatozoa. On the contrary, chicken spermatozoa did not remove this anti-H-Y activity. Moreover, in a quantitative absorption experiment, 2.10^7 human or pig spermatozoa completely removed the anti-H-Y activity of 20 μl of undiluted serum, whereas 10^9 chicken spermatozoa had no effect.

The cytotoxic activity of nonimmune female Lewis rat sera was studied on Raji cells; two out of six sera were found to be cytotoxic on Raji cells at a dilution of 1:2. However, this activity was equally well absorbed by male and female lymphoid cell lines. Several different anti-H-Y sera were analyzed. Sera

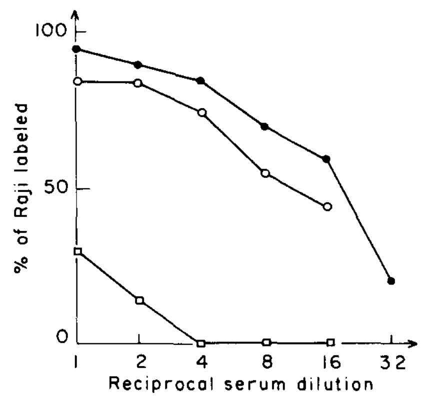

FIG. 4. Presence of H-Y antigen on Raji cells using indirect immunofluorescence test. Curve (●), unabsorbed serum; curve (□), serum absorbed with male (Chevalier) lymphoid line; curve (○), serum absorbed with female (Namalwa) lymphoid line. 50 μl of pure anti-H-Y is absorbed with 2.5 × 10^7 cells.

obtained after weekly injection of 2.10^6 spleen cells had generally a higher activity than those obtained by injecting 4.10^7 cells.

The presence of H-Y antigen on Raji cells could also be demonstrated by indirect immunofluorescence. The anti-H-Y activity was revealed with a fluorescein-conjugated goat anti-rat IgG. 80–90% of cells were labeled (Fig. 4). The labeling was removed after absorption with male lymphoid cells but not with female cells. When these experiments were performed at 37°C, caps were observed on 60% of the labeled cells.

Presence of H-Y Antigen on Different Human Lymphoid Cell Lines. The expression of H-Y antigen was examined on 12 lymphoid lines established from male donors and 10 lymphoid lines from female donors by direct lymphocytotoxic or absorption technique (Table I). None of the 10 female cell lines absorbed the anti-H-Y activity. On the contrary, 10 out of 12 male cell lines expressed H-Y antigen by absorption criteria. Of the H-Y positive cell lines, one, Chevalier, lacks HLA antigens but is β_2-microglobulin positive (Fellous et al. Manuscript in preparation). The two exceptional male lines that lacked H-Y antigen were Ramos (19), which also lacks the Y chromosome, and Daudi, which already lacks HLA and β_2-microglobulin. By direct lymphocytotoxic and indirect immunofluorescence assay, the H-Y antigen was detected on Raji, Chevalier, SKL-1 but not on the other seven male lymphoid lines. It should be noted that the presence of H-Y antigen could be detected only by absorption on most male lymphoid lines, as it has been already found on normal lymphocyte from other species (6). In this context Raji and Chevalier obtained from Burkitt lymphoma seem to be rather exceptional.

Relationship Between the Expression of β_2-Microglobulin and of H-Y Antigen

H/Y ANTIGEN IS ABSENT ON DAUDI CELL BUT IS EXPRESSED AFTER CELL FUSION. The Daudi cell line carries the Y chromosome but lacks the H-Y antigen. It is already known that Daudi lacks the human β_2-microglobulin and HLA antigens (15, 16, 38). It was therefore of particular interest to determine

TABLE I
Expression of H-Y Antigen on Human Lymphoblastoid Lines from Male and Female Donors

Cell lines	Sex chromosomes	Direct cytotoxic test Killed cells	Immunofluorescence test Labeled cells	Absorption* Presence of H-Y antigen
		%	%	
From male donors				
Raji‡	XY	100	98	Present
Chevalier‡	XY	100	70	Present
SKL‖	XY	60	60	Present
JOST§	XY	0	0	Present
ScTa§	XY	0	0	Present
REMB1§	XY	0	0	Present
H2LcL§	XY	0	0	Present
RPMI 8866‖	XY	0	0	Present
PGw1§	XY	0	0	Present
ESw1§	XY	0	0	Present
Daudi‡	XY	0	0	Not present
Ramos‡	XX	0	0	Not present
From female donors				
BJAB, Namalwa‡	XX	0	0	Not present
T51, Brel, SCBMB georget§	XX	0	0	Not present
WT50, Maja, AUR Letule§	XX	0	0	Not present

* Anti-H-Y activity test on Raji cell after absorption on lymphoid line.
‡ Line from Burkitt lymphoma.
§ Line from normal peripheral blood.
‖ Line from leukemic patient.

whether or not the absence of HLA, H-Y, and β_2-microglobulin on Daudi were related (Table II).

Two types of somatic cell hybrids involving Daudi have been analyzed: (*a*) one hybrid (Raji × Daudi) clone which expresses β_2-microglobulin and HLA (17, 39): this hybrid also expresses the H-Y antigen (Table II); (*b*) one hybrid (Daudi × D98 AH-2) clone, D98 AH-2, being a human female cell line. This hybrid, which has a Y chromosome derived from Daudi, expresses both β_2-microglobulin and H-Y antigen.

EXPRESSION OF H-Y ANTIGEN ON MOUSE TERATOCARCINOMA CELL LINES. The mouse embryonal carcinoma lines, PCC7-S and PCC7-S-Aza$^{R}_{1}$ which carry the Y chromosome were studied (Table III). They both lack β_2-microglobulin and the H-Y antigen. After 3–4 wk of culture under conditions allowing in vitro "differentiation," β_2-microglobulin becomes detectable by indirect immunofluorescence on about 1/3 of the cell population (Table III). Absorption experiments also showed the presence of H-Y antigen on these cells. An eventual correlation between the appearance of H-Y antigen and of β_2-microglobulin was investigated by double labeling immunofluorescence experiment (see Materials and Methods). All the cells which expressed H-Y antigen were also found to be β_2-microglobulin positive; the reverse was not true. Similarly, the mouse embryonal carcinoma cell lines F9, PCC4, and LT_1 (Table III), which are XO or

TABLE II
Relationship between the Expression of Human β_2-Microglobulin HLA and H-Y Antigen on Human Cell Lines

Cell line	Sex chromosomes	Presence of		
		HLA	β_2m*	H-Y
Raji‡	XY	+	+	+
Daudi‡	XY	−	−	−
Raji × Daudi§	XY, XY	+	+	+
D 98	XX	+	+	−
Daudi × D 98§	XX, XY	+	+	+
Chevalier‡	XY	−	+	+

* β_2-microglobulin.
‡ Line from Burkitt lymphoma.
§ Hybrid cell.

TABLE III
Presence of H-Y Antigen on Cell Mouse Teratocarinoma Lines

Embryonal carcinoma line	Presence of differentiated type cells	Sex chromosomes	Detectable antigen using		
			Absorption test* H-Y	Immunofluorescence test	
				Mouse β_2-microglobulin, labeled cells	H-Y,* labeled cells
				%	%
F9	No	XO	−	0	0
PCC4	No	XO	−	NT‡	0
LT_1	No	XX	−	NT	0
PCC7-S, 0 *day*	No	XY	−	0	0
PCC7-S, 28 *days*§	Yes	XY	+	35	24
PCC7-S Aza^R_1, 0 *day*	No	XY	−	0	0
PCC7-S Aza^R_1, 24 *days*§	Yes	XY	+	NT	18
PCC3/A/1, 0 *day*	No	XO	−	0	0
PCC3/A/1, 30 *days*	Yes	XO	−	36	0

* Anti-H-Y activity test on Raji cell after absorption on teratocarcinoma line.
‡ Not tested.
§ Differentiation appears under conditions of differentiation in vitro.

XX, lacked both H-Y antigen and β_2-microglobulin. PCC3/A/1 cells which are XO can differentiate in vitro and then express β_2-microglobulin but still do not express H-Y antigen.

RELATION BETWEEN H-Y AND HUMAN β_2-MICROGLOBULIN ON RAJI CELL. Redistribution experiments were performed on Raji cells to analyze whether H-Y and β_2-microglobulin were associated on the cell membrane (Table IV). After the first labeling with rabbit anti-β_2-microglobulin (revealed by fluorescein or rhodamine conjugated anti-rabbit Ig) and incubation at 37°C, capping was observed on 70–80% of the labeled cells. If the second staining was performed at 0°C with anti-H-Y sera (revealed by rhodamine or fluorescein-conjugated anti-mouse Ig), 90% of the cells doubly stained (Fig. 5) showed

TABLE IV

Effect of Redistribution of Human β_2-Microglobulin on the Distribution of H-Y Antigen on Raji Cell

First labeling	Second labeling	Cells labeled for H-Y	Cells labeled for β_2m*	Percent of cells where labeling of human β_2m is aggregated and: H-Y aggregated at the same place	Percent of cells where labeling of human β_2m is aggregated and: H-Y has a diffuse labeling
37°C	*0°C*	%	%		
Rabbit anti-β_2m 1/200 and FITC goat anti-rabbit Ig	Anti H-Y followed by TRITC goat anti-mouse Ig	90	99	92	8
Rabbit anti-β_2m 1/200 and TRITC goat anti-rabbit Ig	Anti-H-Y followed by FITC goat anti-mouse Ig	85	100	96	4
				Percent of cells where labeling for H-Y is aggregated and: β_2m aggregated at the same place	Percent of cells where labeling for H-Y is aggregated and: β_2m has a diffuse labeling
Anti H-Y and FITC goat anti-mouse Ig	Rabbit anti-β_2m and TRITC goat anti-rabbit Ig	87	99	0	100
		Cells labeled for β_2m		Percent of cells where labeling of β_2m FITC is aggregated and: β_2m TRITC aggregated at the same place	Percent of cells where labeling of β_2m FITC is aggregated and: β_2m TRITC has a diffuse labeling
		%			
Rabbit anti-β_2m 1/200 and FITC goat anti-rabbit Ig	Rabbit anti-β_2m 1/50 and TRITC goat anti-rabbit Ig	100		95	5

* *Abbreviations used in this table:* β_2m, $\beta_2$microglobulin; FITC, fluorescein isothiocyanate; TRITC, tetramethyl rhodamine isothiocyanate.

cocapping of β_2-microglobulin and H-Y antigen. However, in 4–8% of the cells the redistribution of β_2-microglobulin was not associated with a correspondent capping of H-Y antigen. These latter results can tentatively be explained by the fact that in 5% of the cells where β_2-microglobulin caps (last line of Table IV), there is still β_2-microglobulin incompletely redistributed and left outside of the caps. No such cocapping between H-Y and β_2-microglobulin was observed when H-Y antigen is the first to be aggregated.

Discussion

As compared with spermatozoa or epidermal cells, the Raji cell line turns out to be a useful target cell for studies of the H-Y antigen (5, 40): it is easy to grow and killable to 100% by rat anti-H-Y sera at a dilution up to 1:8.

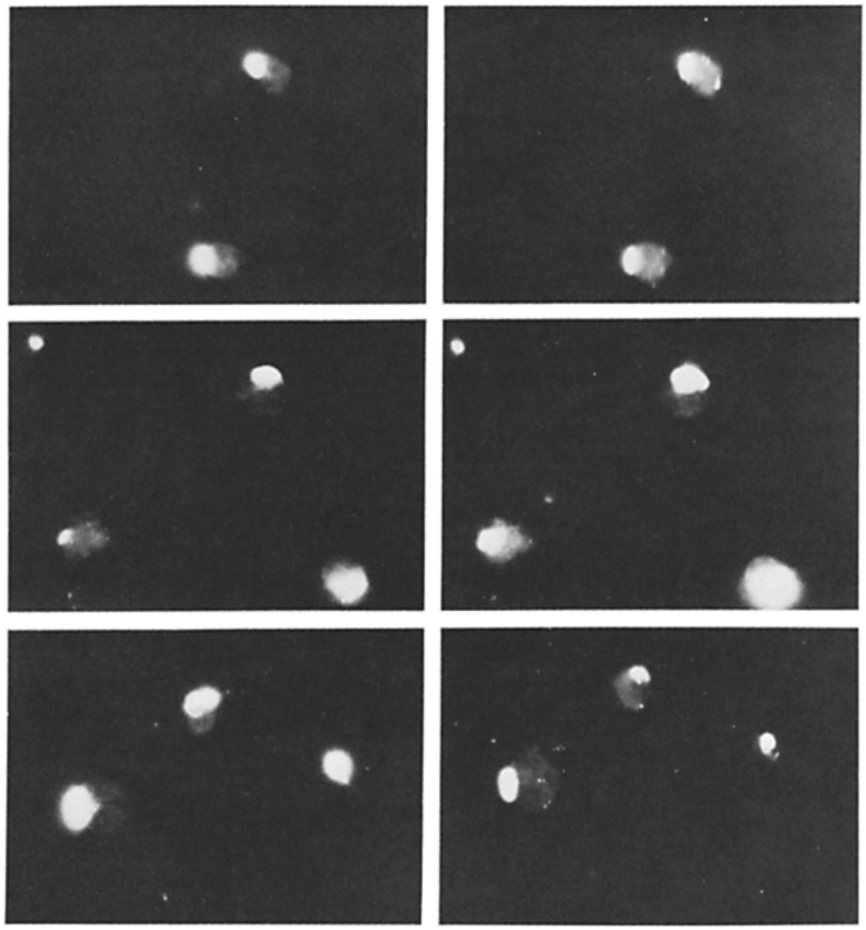

FIG. 5. Redistribution experiment of β_2-microglobulin and H-Y on Raji cells. β_2-Microglobulin were first capped (right panel) with rabbit anti-β_2-microglobulin and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. On the left panel are shown the corresponding cells stained at 4°C with anti-H-Y sera and tetramethyl rhodamine isothiocyanate-conjugated goat anti-mouse IgG. No rhodamine labeling (anti-H-Y) is found outside the green cap (β_2-microglobulin).

Serological screening of male and female human lymphoid cell lines strongly suggests that the serologically detectable H-Y antigen is associated with the presence of the Y chromosome. The H-Y antigen is expressed on all male lymphoid cell lines with only two exceptions: (*a*) the Ramos cell line which was established from a male donor and has lost the Y chromosome in culture, and (*b*) the Daudi cell line which has a Y chromosome. Recently, Ohno (10) has suggested that H-Y antigen used the major histocompatibility β_2-microglobulin antigens complex as an anchorage site on the cell membrane. The experiments reported here were designed to test this hypothesis. The first striking result is the absence of H-Y antigen on Daudi cell, known to lack both β_2-microglobulin and HLA antigens. Cell hybrids between Daudi and D98 (a female cell line that expresses β_2-microglobulin and HLA antigens) express the H-Y antigen. Moreover, Chevalier, a cell line that lacks HLA antigens but expresses β_2-microglobulin, also expresses H-Y antigen.

To test a possible association between HLA, β_2-microglobulin, and H-Y antigen, redistribution experiments between H-Y and β_2-microglobulin or between H-Y and HLA were performed on Raji cells. In the redistribution experiments between β_2-microglobulin and H-Y antigen, 90% cocapping was observed when β_2-microglobulin was redistributed first, but not when the H-Y antigen is capped first. These results show that H-Y antigen is associated with β_2-microglobulin on the cell membrane of Raji. No cocapping has been observed however when redistribution experiments with HLA and H-Y antigens were carried out. These results show clearly that in the cell membrane H-Y antigen is associated only with β_2-microglobulin but not with HLA antigen (as suggested by Ohno).

The association between H-Y antigen and β_2-microglobulin has also been found on mouse teratocarcinoma cells. The male pluripotent embryonal carcinoma lines PCC7-S and PCC7-Aza$^R{}_1$ carrying the Y chromosome lack both H-Y and β_2-microglobulin. During in vitro differentiation correlated appearance of both antigens was observed. Moreover, the cells that are H-Y positive are always β_2-microglobulin positive. On the contrary, β_2-microglobulin positive, H-Y negative cells were found, a result that suggests that during in vitro differentiation β_2-microglobulin might appear before H-Y antigen.

The presence of H-Y antigen has also been described on mouse morulae embryos (41). However, at this stage of embryonic development, we were unable to detect β_2-microglobulin (12). Although the presence of β_2-microglobulin is apparently required for H-Y expression on adult cells, embryonic cells appear to express H-Y without expressing adult β_2-microglobulin. On the embryo, H-Y antigen might be associated with an eventual embryonic form of β_2-microglobulin which has already been postulated (42).

The association between H-Y and β_2-microglobulin must be compared to the already described association between β_2-microglobulin and other membrane antigens related by sequence similarities and coded by closely linked genes: major histocompatibility antigens (37), Tla antigen (43), or Qa antigen (44).

β_2-Microglobulin might behave as a regulatory element in controlling the expression of a series of cell membrane antigens during development and differentiation.

Summary

The expression of the H-Y antigen has been tested on several human lymphoid lines and mouse teratocarcinoma cell lines during differentiation. The human male lymphoid cell line Raji is a very useful target for studies of the H-Y antigen by lymphocytotoxicity test with rat anti-H-Y sera. With a few exceptions, all cells carrying the Y chromosome were H-Y positive. One of the exceptions is the human Daudi cell line which, besides lacking H-Y antigen, also lacks β_2-microglobulin. We have studied a possible association between the H-Y antigen, β_2-microglobulin, and HLA antigen with redistribution experiments. The results strongly suggest that H-Y antigen is not associated with HLA antigens but with β_2-microglobulin.

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References

1. Eichwald, E. J., C. R. Silmser, and I. Weissmann. 1958. Sex-linked rejection of normal and neoplastic tissue. I. Distribution and specificities. *J. Natl. Cancer Inst.* 20:563.
2. Silvers, W. K., and R. E. Billingham. 1967. Genetic background and expressivity of histocompatibility gene. *Science (Wash. D.C.).* 158:118.
3. Gasser, D. L., and W. K. Silvers. 1972. Genetics and immunology of sex-linked antigens. *Adv. Immunol.* 15:215.
4. Wachtel, S. S. 1977. H-Y antigen genetics and serology. *Immunol. Rev.* 33:33.
5. Goldberg, E. H., E. A. Boyse, D. Bennett, M. Scheid, and E. A. Carswell. 1971. Serological demonstration of H-Y (male) antigen on mouse sperm. *Nature (Lond.).* 232:478.
6. Silvers, W. K., and S. L. Yang. 1973. Male specific antigen: its homology in mice and rats. *Science (Wash. D.C.).* 181:570.
7. Wachtel, S. S., G. C. Ko, and E. A. Boyse. 1975. Evolutionary conservation of H-Y (male) antigen. *Nature (Lond.).* 254:270.
8. Gilmour, D. G. 1967. Histocompatibility antigen in the heterogametic sex in the chicken. *Transplantation.* 5:699.
9. Ohno, S. 1976. Major regulatory genes for mammalian sexual development. *Cell.* 7:315.
10. Ohno, S. 1977. The original function of MHC antigens as the general plasma membrane anchorage site of organogenesis-directing proteins. *Immunol. Rev.* 33:59.
11. Appela, E., N. Tanigaki, T. Watanori, and D. Pressman. 1976. Partial amino acid sequence of mouse β_2-microglobulin. *Biol. Biochem. Acta.* 70:425.
12. Dubois, P., M. Fellous, G. Gachelin, F. Jacob, R. Kemler, D. Pressman, and N. Tanigaki. 1976. Absence of a serologically detectable association of human and murine β_2-microglobulin with the F9 antigen. *Transplantation (Baltimore).* 22:467.
13. Fellous, M., A. Colle, and C. Tonnelle. 1976. The expression of human β_2-microglobulin on human spermatozoa. *Eur. J. Immunol.* 6:21.
14. Klein, E., G. Klein, J. S. Nadkarni, J. J. Nadkarni, H. Wigzell, and P. Clifford. 1968. Surface IgM kappa specificity on a Burkitt lymphoma cell in vivo and in derived culture lines. *Cancer Res.* 28:1300.
15. Jones, E. A., P. N. Goodfellow, J. G. Bodmer, and W. F. Bodmer. 1975. Serological identification of HLA linked human Ia type antigen. *Nature (Lond.).* 256:650.
16. Reisfeld, R. A., E. D. Sevies, M. A. Pellegrino, S. Ferrone, and M. D. Poulik. 1975. Association of HLA antigens and β_2-microglobulin at the cellular level. *Immunogenetics.* 2:184.
17. Fellous, M., K.Kamoun, J. Wiels, J. Dausset, G. Clements, J. Zeuthen, and G. Klein. 1977. Induction of HLA expression in Daudi cells after cell fusion. *Immunogenetics.* 5:423.
18. Pulvertaft, R. J. V. 1965. A study of malignant tumor in Nigeria by short term tissue culture. *J. Clin. Pathol. (Lond.).* 18:261.
19. Klein, G., J. Zeuthen, P. Terasaki, R. Billing, R. Honig, M. Jondal, A. Westmas, and G. Clements. 1976. Inducibility of the Epstein-Barr virus cycle and surface marker properties of EBV negative lymphoma and their in vitro EBV converted sublines. *Int. J. Cancer.* 18:639.

20. Klein, G., T. Lindahl, M. Jondal, W. Leibold, J. Menezes, K. Nilsson, and C. Sundström. 1974. Continuous lymphoid cell lines with characteristics of B cells, lacking the Epstein-Barr virus genome and derived from three human lymphomas. *Proc. Natl. Acad. Sci. U. S. A.* **71**:3283.

21. Klein, G., L. Dombos, and B. Gothoskar. 1972. Sensitivity of Epstein-Barr virus producer and non producer human lymphoblastoid cell lines to superinfection with EB virus. *Int. J. Cancer.* **10**:44.

22. Clarkson, B., A. Striss, and E. De Harven. 1967. Continuous culture of seven new lines (SKL-1 to 7) from patients with acute leukemia. *Cancer.* **20**:920.

23. Steel, C. M., and E. Edmond. 1971. Human lymphoblastoid cell lines. I. Culture methods and examination for Epstein-Barr virus. *J. Natl. Cancer Inst.* **47**:1193.

24. Sly, W. S., G. S. Sekhon, R. Kennett, W. F. Bodmer, and J. G. Bodmer. 1975. Permanent lymphoid lines from genetically marked lymphocytes: success with lymphocytes recovered from frozen storage. *Tissue Antigens.* **40**:155.

25. Clements, G., G. Klein, J. Zeuthen, and S. Povey. 1976. The selection of somatic cell hybrids between human lymphoma cell lines. *Somatic Cell Genet.* **2**:309.

26. Kennett, R. H., B. Hampshire, B. Bengtsson, and W. F. Bodmer. 1975. Expression and segregation of HLA antigens in D98/AH-2 by lymphocyte and fibroblast hybrids. *Tissue Antigens.* **6**:80.

27. Gartler, S. M. 1968. Apparent Hela cell contamination of human heteroploid cell lines. *Nature (Lond.).* **217**:750.

28. Littlefield, J. W. 1964. Selection of hybrids from matings of fibroblasts. *Science (Wash. D.C.).* **145**:709.

29. Borgaonkar, D. S., and D. H. Hollander. 1970. Quinacrine fluorescence of the human Y chromosome. *Am. J. Hum. Genet.* **22**:23*a* (Abstr.).

30. Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* **75**:304.

31. Nicolas, J. F., P. Dubois, H. Jakob, J. Gaillard, and F. Jacob. 1975. Tératocarcinome de la souris: différentiation en culture d'une lignée de cellules primitives à potentialités multiples. *Ann. Microbiol. (Paris).* **126A**:3.

32. Artzt, K., P. Dubois, D. Bennett, H. Condamine, C. Babinet, and F. Jacob. 1973. Surface antigens common to mouse cleavage embryos and primitive teratocarcinoma cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* **70**:2988.

33. Guenet, J. L., H. Jakob, J. F. Nicolas, and F. Jacob. 1974. Tératocarcinome de la souris: étude cytogénétique de cellules à potentialités multiples. *Ann. Microbiol. (Paris).* **125A**:10.

34. Nicolas, J. F., P. Avner, J. Gaillard, H. Jakob, and F. Jacob. 1976. Cell lines derived from teratocarcinomas. *Cancer Res.* **36**:4224.

35. Fellous, M., G. Gachelin, M. H. Buc-Caron, P. Dubois, and F. Jacob. 1974. Similar location of an early embryonic antigen on mouse and human spermatozoa. *Dev. Biol.* **41**:331.

36. Mittal, K. K. 1977. Standardization of the HLA typing method and reagents. *Vox Sang.* **34**:58.

37. Neauport-Sautes, C., A. Bismuth, F. M. Kourilsky, and Y. Manuel. 1974. Relationship between HLA antigens and β_2-microglobulin as studied by immunofluorescence on the lymphocyte membrane. *J. Exp. Med.* **139**:957.

38. Evrin, P. E., and K. Nilsson. 1974. β_2-Microglobulin production in vitro by human hematopoietic, mesenchymal land epithelial cells. *J. Immunol.* **112**:137.

39. Klein, G., P. Terasaki, R. Billing, R. Honing, M. Jondal, A. Rosen, J. Zeuthen, and G. Clements. 1977. Somatic cell hybrids between human lymphoma cell lines. III. Surface markers. *Int. J. Cancer.* **19**:66.

40. Scheid, M., E. A. Boyse, E. A. Carswell, and L. J. Old. 1972. Serologically demonstrable alloantigens of mouse epidermal cells. *J. Exp. Med.* **135**:938.
41. Krco, C. J., and E. H. Goldberg. 1976. H-Y antigen: detection on eight-cell mouse embryos. *Nature (Lond.)*. **193**:1134.
42. Vitetta, E. S., K. Artz, D. Bennett, E. A. Boyse, and F. Jacob. 1975. Structural similarities between a product of the T/t locus from sperm and teratoma cells and H-2 antigens isolated from splenocytes. *Proc. Natl. Acad. Sci. U. S. A.* **72**:3215.
43. Vitetta, E. S., J. W. Uhr, and E. A. Boyse. 1975. Association of a β_2-microglobulin-like subunit with H-2 and TL alloantigens on murine thymocytes. *J. Immunol.* **114**:252.
44. Michaelson, J., L. E. Flaherty, E. Vitetta, and M. D. Poulik. 1977. Molecular similarities between the Qa-2 alloantigens and other gene products of the 17th chromosome of the mouse. *J. Exp. Med.* **145**:1066.