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# Human transferrin receptor: Expression of the receptor is assigned to chromosome 3

(gene mapping/comparison of human and mouse transferrin receptors/radioimmunoassay/mouse–human lymphocyte hybrid)

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**ABSTRACT** **Human chromosome 3 has been identified as responsible for expression of the transferrin receptor in mouse–human lymphocyte hybrids. The receptor was detected by immunoprecipitation with anti-human receptor antibody of $^{125}$I-labeled cells. This method also detected a similar 94,000-dalton protein in mouse cells. A radioimmunoassay developed for the human transferrin receptor measured 10% crossreactivity with the mouse protein. The two proteins were distinguished by NaDodSO$_4$/polyacrylamide gel patterns of partial proteolytic digests of the immunoprecipitated proteins. Mouse–human hybrids were generated by fusing a mouse thymoma (BW5147) cell line to either concanavalin A- or pokeweed mitogen-activated human peripheral blood lymphocytes or a mouse myeloma (NS-1) to uncultured human peripheral blood lymphocytes. Each hybrid was karyotyped with respect to both mouse and human chromosomes. In every case, expression of the human transferrin receptor correlated only with human chromosome 3.**

The genes for a number of human cell surface proteins have been assigned to specific chromosomes and chromosome regions by studying somatic cell hybrids between human and rodent cells (1–4). Several of these genes have been assigned to the human X chromosome (1, 2, 5–7); others have been assigned to the autosomes (3, 4, 8–11).

In most cases, the antigens have been defined only by reactivity with human cells and mouse–human hybrid clones containing certain human chromosomes (1, 5, 10) although, in a few, the marker molecules have been characterized as well (7, 9).

In this paper, we describe expression of the human transferrin receptor in mouse–human lymphocyte cell hybrids and assign the gene for this receptor to human chromosome 3. Assignment is based on identification of the transferrin receptor by its molecular and immunochemical properties. Partial digestion patterns were used to distinguish the human transferrin receptor from the mouse transferrin receptor. We have also identified and characterized molecular and immunochemical properties of the mouse transferrin receptor.

## MATERIALS AND METHODS

**Fusion and Culture of Cells.** A hypoxanthine/aminopterin/thymidine-sensitive AKR mouse thymoma (BW5147) was fused in polyethylene glycol 1500 with either concanavalin A (Con A) (hybrids 7–28)- or pokeweed mitogen (PWM) (hybrids 1–5)-activated human peripheral blood lymphocytes. The hybrids were isolated in selective culture medium [Dulbecco's modified Eagle's medium (GIBCO)/15% newborn calf serum (GIBCO)/0.1 mM hypoxanthine/0.4 μM aminopterine/13 μM thymidine containing penicillin at 100 μg/ml and streptomycin at 100 units/ml] and cloned by limiting dilution on human fibroblast feeders. A hypoxanthine/aminopterin/thymidine-sensitive BALB/c myeloma (NS-1) was fused with human peripheral blood lymphocytes, and the hybrids were cultured and cloned as T-cell hybrids.

The hybrid clones and the cell lines were cultured in Dulbecco's modified Eagle's medium/15% newborn calf serum, the human erythroid cell line K562 was cultured in RPMI 1640 (GIBCO)/10% fetal calf serum, and BeWo cells were cultured in BeWo medium (12). All cultures were grown in the presence of penicillin (100 μg/ml) and streptomycin (100 units/ml) (GIBCO).

**Karyotypes and Clones.** Chromosome preparations were made from the hybrid clones on multiple occasions (see Table 2) and once from the BW5147 and NS-1 cell lines. Giemsa bands were obtained with two different protocols: (*i*) a modification of the trypsin Giemsa banding method (13) that allows discrimination between mouse and human chromosomes due to G bands and dark centromere staining of mouse, but not human, chromosomes (Fig. 1) and (*ii*) G-11 banding (14) that stains most human chromosome regions faint blue and all mouse chromosome regions, except the centromere, dark red-blue. On each occasion, slides were screened for human chromosomes after G-11 staining and 20 G-banded cells were analyzed in detail.

**Iodination and Immunoprecipitation.** Cells were grown at 2–5 × 10$^5$/ml, washed three times by centrifugation at 2,000 × *g* for 5 min, and suspended in phosphate-buffered saline (10 mM sodium phosphate/0.15 M NaCl, pH 7.4). They (1 × 10$^7$ cells) were then iodinated with Na$^{125}$I (New England Nuclear) by a lactoperoxidase-catalyzed reaction and washed free of unbound iodine as described (15). This was followed by solubilization in 2 ml of 10 mM Tris·HCl/0.15 M NaCl/5 mM EDTA, 1% Triton X-100, pH 7.2, and centrifugation at 70,000 × *g* at 4°C for 0.5 hr to pellet undissolved material. Solubilized samples, containing 2–10 × 10$^6$ cpm of radioactivity, were first preadsorbed with nonimmune goat serum and *Staphylococcus aureus* (Calbiochem) for 1 hr at 4°C. After centrifugation (1 min, 12,000 × *g*), the supernatants were immunoprecipitated with goat antiserum to the human transferrin receptor. This antibody has been described and characterized (16). The immune complex was incubated 1 hr or overnight at 4°C and extensively washed with 10 mM Tris·HCl/0.15 M NaCl/5 mM EDTA/0.1% Triton X-100, pH 7.2/5 mM NaI containing ovalbumin

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Abbreviations: PWM, pokeweed mitogen; Con A, concanavalin A.

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at 1 mg/ml. The pellet was eluted with 100 μl of 1.5% $NaDodSO_4$/0.08 M Tris·HCl, pH 6.8/5% glycerol.

**$NaDodSO_4$/Polyacrylamide Gel Electrophoresis.** Electrophoresis was carried out on 8% acrylamide gels by the method of Laemmli (17) with modifications by Wada *et al.* (15). Samples eluted from immunoprecipitates were reduced with 5% 2-mercaptoethanol and boiled for 1.5 min prior to electrophoresis.

**Digestion with *S. aureus* Protease and Peptide Mapping.** The $^{125}I$-labeled material eluted from the immunoprecipitates was loaded onto a 4% stacking gel on top of a 13% polyacrylamide separating gel. Digestion with *S. aureus* protease (Miles) and electrophoresis to separate the resulting peptides followed the method of Cleveland *et al.* (18).

**Radioimmunoassays.** The radioimmunoassay for the transferrin receptor has been described (16). Briefly, purified transferrin receptor from human placentae and antibody generated in goats against the receptor are used. The procedure described was modified by using rabbit anti-goat immunobeads (250 μl per tube; Bio-Rad) instead of a second antibody to separate the labeled antigen–antibody complex from unbound labeled antigen.

## RESULTS

**Establishment of Hybrid Cell Lines.** Mouse–human hybrids were generated by fusing a hypoxanthine/aminopterin/thymidine-sensitive mouse thymoma cell line, BW5147, with activated human peripheral blood lymphocytes, and a similarly sensitive mouse myeloma cell line, NS-1, with uncultured human peripheral blood lymphocytes. The activated human lymphocytes were either Con A-stimulated (clone series 7–28) or PWM stimulated (clone series 1–5). The clones were established by limiting dilution.

**Identification of the Human Transferrin Receptor in Mouse–Human Hybrid Clones.** The initial procedure for screening the hybrid clones for the human transferrin receptor used immunoprecipitation of $^{125}I$-labeled cell surface membranes with a goat anti-human transferrin receptor IgG preparation. The karyotype of the clones varies from cell to cell. However, the set of normal mouse chromosomes and mouse marker chromosomes present in the mouse parental cell lines can be identified in all cells. Human chromosomes are not stable in these hybrid clones and are lost with time in culture. Because of this, the karyotypes were analyzed simultaneously with immunoprecipitation of the membranes for electrophoretic analysis. The percentages of human chromosomes in each clone are given in Tables 1–3. The data from the hybrids between mouse cells and human Con A- or PWM-activated lymphocytes show that expression of the human transferrin receptor is always associated with the presence of human chromosome 3 (Tables 1 and 2). Furthermore, the absence of this receptor is always associated with the absence of this chromosome. However, in early cultures (Table 1), all clones expressing the human transferrin receptor also contained the human X chromosome as well as human chromosomes 6 and 21, while the clones carrying human chromosomes X, 6, or 21 but not human chromosome 3 never expressed the human transferrin receptor. After prolonged culture, one clone carrying only human chromosomes X, 3, and 21 and one clone carrying only chromosomes X and 3 expressed the human transferrin receptor (Table 2). Furthermore, a complete correlation was found between the loss of human chromosomes 3 and X with time in culture and simultaneous loss of the human transferrin receptor (Table 2). Karyotypes of cells from human transferrin receptor-positive and -negative clones are shown in Fig. 1. These experiments do not rule out the possibility of human chromosome X participating in transferrin receptor expression. This, in fact, is not the case, as we have studied two mouse–human B-cell clones (25 and 73) lacking the human X chromosome and one containing human chromosome 3 (see Fig. 2*B*). The one carrying chromosome 3 was positive for the human transferrin receptor; the one lacking it was negative (Table 3).

**Distinction of Human Transferrin Receptor from Mouse Transferrin Receptor.** Close examination of the $NaDodSO_4$/polyacrylamide gels reveals a faint band that travels at the same molecular weight as the human transferrin receptor in the neg-

Table 2. Loss of human chromosomes with time in culture

| Hybrid | % mitosis containing human chromosome | | | | | | | Time in culture, wk | Transferrin receptor reactivity* |
|---|---|---|---|---|---|---|---|---|---|
| | X | 3 | 6 | 7 | 8 | 11 | 21 | | |
| 1-1 | 65 | 40 | 40 | 30 | 5 | 10 | 15 | 1 | ++ |
| 1-1 | 10 | 5 | | | | | | 11 | + |
| 1-3 | 50 | 60 | 20 | | | | 15 | 1 | ++ |
| 1-3 | 5 | 15 | | | | | 10 | 4 | + |
| 1-3 | | | | | | | | 11 | − |

The presence of the transferrin receptor was determined by immunoprecipitation of $^{125}I$-labeled cells followed by $NaDodSO_4$/polyacrylamide gel electrophoresis (see Fig. 2).
* ++, Very reactive; +, reactive; −, not reactive.

Table 1. Karyotypic analysis of human chromosomes in mouse–human clones

| Hybrid | % mitosis containing human chromosome | | | | | | | | | | | | | | | Cell stimulation | Transferrin receptor* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 11 | 12 | 14 | 16 | 17 | 19 | 21 | | |
| 1-1 | 65 | 40 | | | 40 | 30 | 5 | | 10 | | | | | | 15 | PWM | + |
| 1-3 | 50 | 60 | | | 20 | | | | | | | | | | 15 | PWM | + |
| 2-3 | 25 | 30 | | | 55 | | | | | | | | | | 55 | PWM | + |
| 28-5 | 75 | 75 | | 25 | 75 | | | | | | | | | | 40 | Con A | + |
| 1-2 | 85 | | | | | | | | | | | 5 | | | | PWM | − |
| 5-1 | 40 | | | | | | | | | | | | | | 20 | PWM | − |
| 7-1 | | | | | 85 | | | | | | | | | | | Con A | − |
| 7-18 | | | | | 50 | | | 5 | | | | | | | | Con A | − |
| 13-4 | 90 | | | | 70 | | | | | | | | | | | Con A | − |
| 13-19 | 5 | | | | 20 | | | | | | | | | | | Con A | − |
| 28-3 | 70 | | | 70 | 55 | 50 | | 70 | 10 | | 25 | | | | 50 | Con A | − |
| 28-4 | 80 | | 60 | 70 | 100 | | | 70 | 80 | 20 | | | 20 | 10 | 70 | Con A | − |

Chromosomes were analyzed within 3 days of electrophoresis with the exception of hybrids 13-4 and 28-4, which were analyzed only early in culture. The presence of the transferrin receptor was determined by immunoprecipitation of $^{125}I$-labeled cells followed by $NaDodSO_4$/polyacrylamide gel electrophoresis (see Fig. 2).
* +, Present; −, absent.

Table 3. Karyotypic analysis of human chromosomes in mouse–human B-cell clones

| Hybrid | % mitosis containing human chromosome | | | | | | | | | | | | | | | | | | | | | | Human transferrin receptor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | |
| 25 | – | 70 | – | 10 | 20 | 50 | – | 90 | 40 | 60 | – | – | 10 | – | 50 | 60 | – | 60 | 30 | – | 10 | 40 | – | + |
| 73 | – | 10 | – | – | – | 20 | – | 20 | – | – | – | – | – | – | 20 | – | – | 10 | – | – | 10 | 50 | – | – |

The mouse–human B-cell hybrids were produced by fusion of activated human peripheral blood lymphocytes and a mouse myeloma (NS-1). Both clones tested secrete human immunoglobulin heavy chain (m$\mu$). +, Present; –, absent.

ative hybrids as well as the mouse parent cell line. Nonspecific binding of a $M_r$ 94,000 protein to *S. aureus* was ruled out, as shown in Fig. 2*A*. Eluates of the *S. aureus* used to preadsorb the solubilized sample show no detectable radioactive bands. Thus, it appears that not only is there some crossreactivity between the mouse and human transferrin receptor but the receptors have indistinguishable mobilities in denaturing Na-Dod$SO_4$ gels. The degree of crossreactivity was quantitated by measuring the amount of receptor present by using a radioimmunoassay for the human transferrin receptor. The results are summarized in Table 4. The amount of transferrin receptor in the hybrid clones (1-1 and 1-3) is less than that in activated peripheral blood lymphocytes. This is consistent with the hybrids containing 40–50% human chromosome 3. No significant differences between the negative clones and the mouse parent cell lines were seen. These values are 1/6 to 1/7 of those for the positive cell lines, in which ≈50% of the cells contain human chromosome 3 by karyotypic analysis. From this, we estimate that there is <10% crossreactivity between mouse and human transferrin receptors.

To exclude the possibility that human chromosome 3 could be stimulating expression of the mouse transferrin receptor that crossreacts with the anti-human transferrin receptor antiserum, we used partial proteolytic digestion patterns (16) to examine the proteins immunoprecipitated from human, mouse–human, and mouse cell lines. As shown in Fig. 3, protease digests of the transferrin receptors from mouse and human cell lines are different. Fig. 3*A* shows that the mouse–human hybrid digestion pattern for clone 1-3, which is positive for human chromosome 3, is a combination of the patterns of the receptors isolated from BW5147 cells and a human cell line, BeWo. This analysis was carried out after clone 1-3 had been 4 weeks in culture and ≈15% of the cells still retained human chromosome 3. Thus, the relative intensities (radioactivity precipitated) are similar. The clone shown in Fig. 3*B*, however, was immunoprecipitated when 40% of the cells carried human chromosome 3, and so different exposure times of the immunoprecipitates were required to compare the cell lines. Again, the digestion pattern of the clone containing human chromosome 3 (clone 1-1) appears to be a composite of the parent mouse cell line and a human cell line (K562). In contrast, the digestion pattern of 13-19 containing no human chromosome 3 is pure mouse in character. The proteolytic digestion patterns of the two human cell lines showed no significant differences from gel to gel. These cells are of diverse origin, K562 being an erythroid myeloid cell line and BeWo being a choriocarcinoma cell line. Previous studies have shown that the transferrin receptors isolated from various human tissues are identical on partial proteolytic digestion maps, suggesting all are the same molecule (19).

## DISCUSSION

The transferrin receptor is a cell surface glycoprotein that has been identified on cells in culture, cells of erythroid origin, and placentae. In addition, it has been postulated to be a marker of cell proliferation (20–22). Low but detectable levels of transferrin receptor have been identified in normal peripheral lymphocytes (23, 24) and may be from the small number of blast cells present. Mitogen activation of peripheral lymphocytes with Con A or phytohemagglutinin increases the amount of

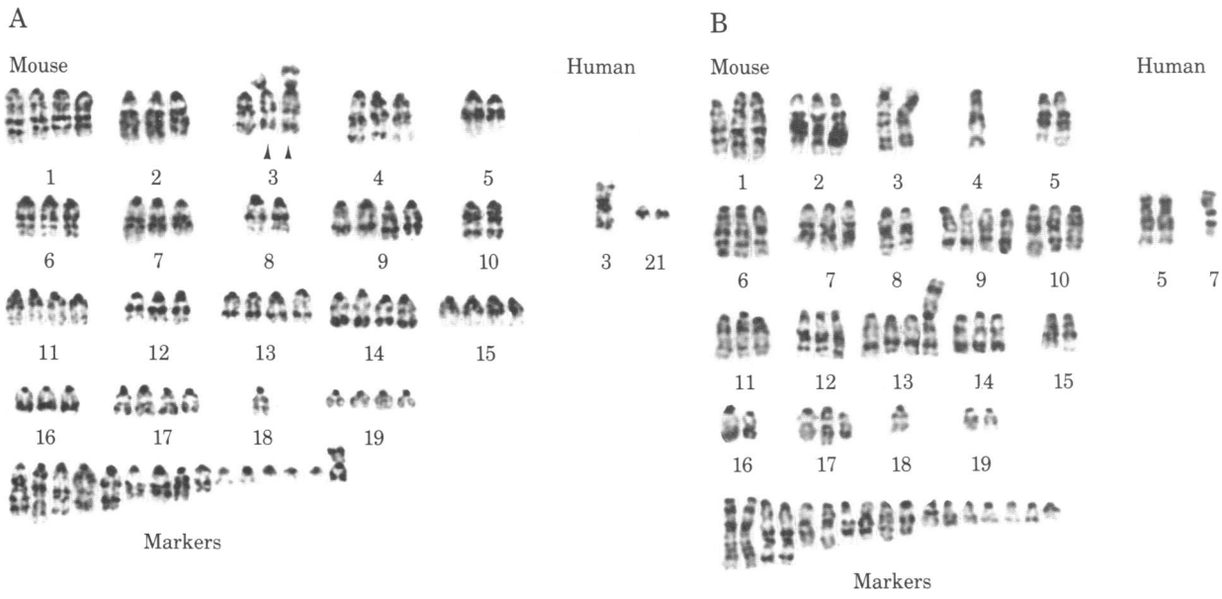


FIG. 1. Karyotypes of mouse–human hybrid clones. (*A*) Cell from clone 1-1 carrying one human chromosome 3 and two copies of human chromosome 21. Abnormal copies of mouse chromosome 3 are indicated by arrowheads. (*B*) Cell from clone 28-3 carrying two copies of human chromosome 5 and one human chromosome 7.

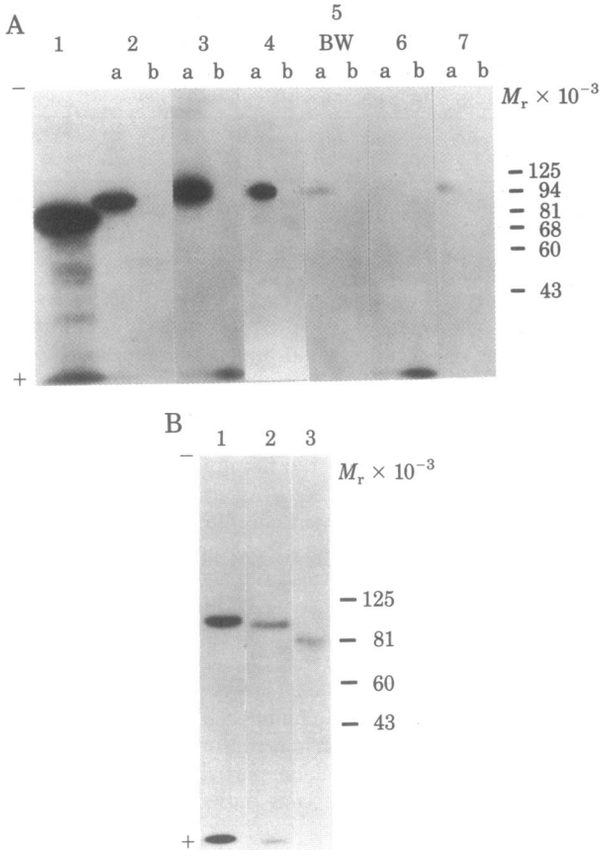


FIG. 2. Autoradiograms of $NaDodSO_4$/8% polyacrylamide gel electrophoresis of immunoprecipitates from $^{125}I$-labeled cell lines. Cell lines were iodinated and immunoprecipitated with antiserum to the human transferrin receptor and *S. aureus*. (*A*) Eluates of immunoprecipitates from mouse and human cell lines and hybrid clones. Lanes: 1, $^{125}I$-labeled human transferrin (Sigma) used as a standard; 2, K562, a human erythroid myeloid cell line; 3 and 4, mouse–human hybrids 1-1 and 1-3, which possess human chromosome 3; 5, parent mouse cell line BW5147; 6 and 7, mouse–human hybrids 13-19 and 5-1, which do not possess human chromosome 3. a, Eluate from specific immunoprecipitation with goat anti-human transferrin receptor serum and *S. aureus*; b, eluate from preadsorption with nonimmunized goat serum and *S. aureus*. (*B*) Eluates of immunoprecipitates from a mouse–human B-cell hybrid clone and a mouse cell line. Lanes: 1, a mouse–human B-cell hybrid 25 with 10% of the cells containing human chromosome 3; 2, a mouse cell line (BW5147); 3, $^{125}I$-labeled human transferrin as a standard. Both cell lines were iodinated to the same extent ($3 \times 10^6$ cpm) and immunoprecipitated in an identical manner. $M_r$ markers run simultaneously and stained with Coomassie blue were *Escherichia coli* β-galactosidase (Sigma), 94,000; human transferrin (Sigma), 81,000; bovine serum albumin (Sigma), 68,000; beef liver catalase (Boehringer Mannheim), 60,000; chicken egg ovalbumin (Sigma), 43,000.

transferrin receptor detected by transferrin binding (24, 25). Although the mechanism responsible for this increase in measurable binding is not known, we have exploited this observation to look for the human chromosome associated with expression of the transferrin receptor. To accomplish this, we generated mouse–human hybrids by using a mouse thymoma cell line (BW5147) and mitogen-activated human lymphocytes from normal volunteers.

**Assignment of the Transferrin Receptor to Human Chromosomes.** The association we found between the expression of the human transferrin receptor and chromosome 3 was strong. Not a single clone lacking human chromosome 3 was positive for the receptor, while all clones carrying chromosome 3 expressed the receptor. The correlation between loss of the transferrin receptor and loss of human chromosome 3 with time in culture was complete. No such correlation was found between expression of the human transferrin receptor and the presence of human chromosomes 6 and 21, which in early cultures were present in clones positive for the receptor.

Table 4. Transferrin receptor values

| | Value, ng per $2 \times 10^7$ cells | % human chromosome 3 |
|---|---|---|
| Clone | | |
| 1-1 | 325 ± 30 | 40 |
| 1-3 | 348 ± 30 | 60 |
| 5-1 | 60* ± 20 | — |
| 13-19 | 60* ± 20 | — |
| BW5147 cells | 40* ± 20 | |
| Con A PBL | 830 ± 30 | |

Values were measured by a radioimmunoassay. Con A PBL, Con A-activated human peripheral blood lymphocytes.
* Limits of detectability of the radioimmunoassay.

Although all clones positive for the receptor carried the human X as well, it is unlikely that this chromosome is involved in expression of the human transferrin receptor, since none of the clones carrying this chromosome but lacking chromosome 3 were positive. This is further supported by study of mouse–human B-lymphocyte hybrids that do not have the human X chromosome but carry 3 and express the human transferrin receptor. Our data have been independently confirmed by Goodfellow *et al.* (§), who showed expression of the human transferrin receptor correlated with human chromosome 3 by using a monoclonal antibody to the receptor, OKT-9, in mouse–human hybrids.

**Identification of the Human Transferrin Receptor.** To distinguish between mouse and human transferrin receptors, antiserum to purified human transferrin receptor was used. This was done for a variety of reasons. Specific binding of human transferrin has been measured in a mouse teratocarcinoma stem cell line by Karrin and Mintz (26), in a mouse erythroleukemia cell line by Wilczynska and Schulman (27), and in a variety of human tissues and cells. Since the binding constant measured for the human transferrin–mouse receptor interaction on teratocarcinoma cells ($K_a = 1.49 \times 10^8\ M^{-1}$) was no weaker than binding constants for human transferrin–human receptor interactions ($K_a = 10^7$–$10^9\ M^{-1}$) (15, 23, 28, 29), it is not a definitive criterion for identifying human transferrin receptors in mouse–human hybrids. In addition, transferrin has been identified as being a requirement for growth of cells in culture (30). This makes it unlikely that one could select a mouse mutant lacking transferrin receptor.

The antiserum generated against the human transferrin receptor enabled us to distinguish between the species' specific phenotypes. Although there was some degree of crossreactivity (≈10%) between human and mouse transferrin receptors, we could distinguish between them quantitatively by using a radioimmunoassay and definitively by using partial proteolytic digestion patterns.

Human transferrin has been shown to specifically bind to the surface membranes of a variety of intergeneic animal species (including rat, mouse, and rabbit) (26, 31, 32). Not only must the binding sites be similar but, as we have shown, there is some degree of immunological crossreactivity and a striking similar-

§ Goodfellow, P. N., Banting, G., Sutherland, R., Greaves, M., Solomon, E. & Povey, S. *Abstracts of Sixth International Workshop on Human Gene Mapping*, Oslo, Norway, June 29–July 3, 1981. (F. H. Ruddle, personal communication.)

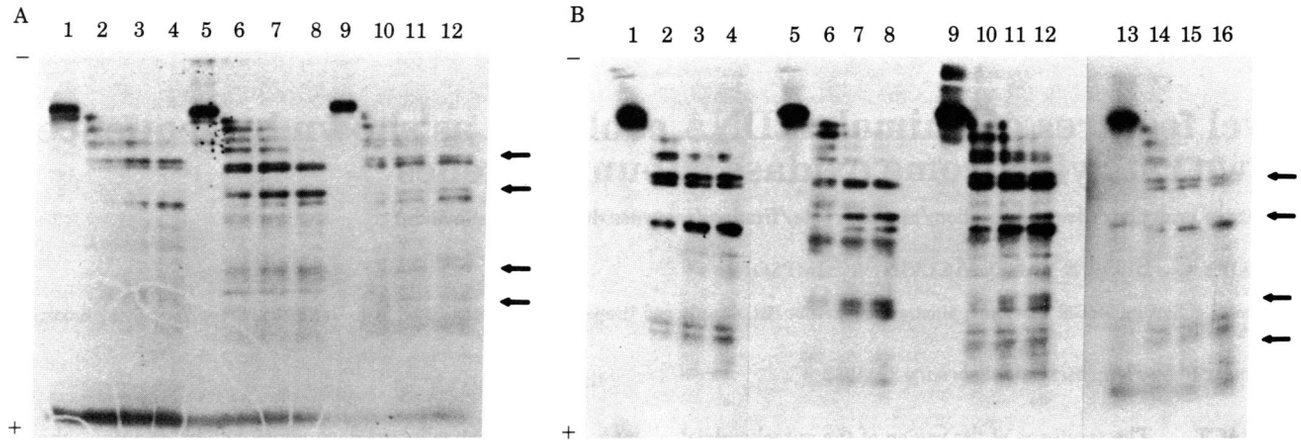


FIG. 3. Digestion patterns of eluates of antitransferrin receptor immunoprecipitates from mouse, human, and mouse–human hybrid clones. Iodinated cell lines were immunoprecipitated with antitransferrin receptor IgG, washed, and eluted with $NaDodSO_4$. Eluates of each cell line were loaded into each of four wells and *S. aureus* protease at 12.5 mg (lanes 2, 6, 10, and 14), 25 mg (lanes 3, 7, 11, and 15), or 50 mg (lanes 4, 8, 12, and 16) was layered on each set of samples. Lanes: 1, 5, 9, and 13, no *S. aureus*. (*A*) Lanes: 1–4, mouse cell line BW5147 (1,500 cpm per well); 5–8, human cell line BeWo (2,400 cpm per well); 9–12, mouse–human hybrid clone 1-3 containing chromosome 3 in 15% of the cells (1,250 cpm per well). X-Omat film (Kodak) was exposed 9 days at −76°C. (*B*) Lanes: 1–4, mouse cell line BW5147 (800 cpm per well); 5–8, K562, a human erythroid myeloid cell line (2,500 cpm per well); 9–12, mouse–human hybrid 1-1 with 40% of the cells containing human chromosome 3 (3,300 cpm per well); 13–16, mouse–human hybrid 13-19 lacking human chromosome 3. Film was exposed 5 days at −76°C for the K562 and 1-1 cells and 20 days for 13-19 and BW5147 cells. Arrows indicate major differences between mouse and human transferrin receptor proteolytic digestion patterns.

ity in the proteolytic digestion patterns of the crossreactive materials.

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1. Buck, D. W., Goss, S. J. & Bodmer, W. F. (1976) *Cytogenet. Cell Genet.* **16**, 99–100.
2. Dorman, B. P., Shimizu, N. & Ruddle, F. H. (1978) *Cytogenet. Cell Genet.* **22**, 243–244.
3. Chan, M. M., Kano, K., Dorman, B., Ruddle, F. H. & Milgrom, F. (1979) *Immunogenetics* **8**, 265–275.
4. Owerbach, D., Doyle, D. & Shows, T. B. (1979) *Somat. Cell Genet.* **5**, 281–301.
5. Dorman, B. P., Shimizu, N. & Ruddle, F. H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2363–2367.
6. Schwab, R. & Siniscalco, M. (1978) *Cytogenet. Cell Genet.* **22**, 538–540.
7. Goodfellow, P., Banting, G., Levy, R., Povey, S. & McMichael, A. (1980) *Somat. Cell Genet.* **6**, 777–787.
8. Knowles, B. B., Mausner, R. & Aden, D. P. (1978) *Cytogenet. Cell Genet.* **22**, 250–254.
9. Jones, C., Moore, E. E. & Lehman, D. W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6491–6495.
10. Shimizu, N., Behzadian, M. A. & Shimizu, Y. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3600–3604.
11. Davies, R. L., Grosse, V. A., Kucherlapati, R. & Bothwell, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4188–4192.
12. Pattillo, R. A. & Gey, G. O. (1968) *Cancer Res.* **28**, 1231–1236.
13. Seabright, M. (1971) *Lancet* ii, 971–972.
14. Wyandt, H. E., Wysham, D. G., Minden, S. K., Anderson, R. S. & Hecht, F. (1976) *Exp. Cell Res.* **102**, 85–94.
15. Wada, H. G., Hass, P. E. & Sussman, H. H. (1979) *J. Biol. Chem.* **254**, 12629–12635.
16. Enns, C. A., Shindelman, J. E., Tonik, S. E. & Sussman, H. H. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4222–4225.
17. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
18. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106.
19. Enns, C. A. & Sussman, H. H. (1981) *J. Biol. Chem.* **256**, 12620–12623.
20. Hamilton, T. A., Wada, H. G. & Sussman, H. H. (1979) *J. Supramol. Struct.* **11**, 503–515.
21. Shindelman, J. E., Ortmeyer, A. E. & Sussman, H. H. (1981) *Int. J. Can.* **27**, 329–334.
22. Trowbridge, I. S. & Omary, M. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3040–3043.
23. Hamilton, T. A., Wada, H. G. & Sussman, H. H. (1980) *Scand. J. Immunol.* **11**, 195–201.
24. Larrick, J. W. & Cresswell, P. (1979) *J. Supramol. Struct.* **11**, 579–586.
25. Galbraith, G. P. & Galbraith, R. M. (1980) *Clin. Exp. Immunol.* **42**, 285–293.
26. Karrin, M. & Mintz, B. (1981) *J. Biol. Chem.* **256**, 3245–3252.
27. Wilczynska, A. & Schulman, H. M. (1980) *Can. J. Biochem.* **58**, 935–940.
28. Loh, T. T., Higuchi, D. A., van Bockxmeer, F. M., Smith, C. H. & Brown, E. B. *J. Clin. Invest.* **65**, 1182–1191.
29. Galbraith, R. M., Werner, P., Arnaud, P. & Galbraith, G. M. P. (1980) *J. Clin. Invest.* **66**, 1135–1143.
30. Barnes, D. & Sato, G. (1980) *Cell* **22**, 649–655.
31. Ecarot-Charrier, B., Grey, V. L., Wilczynska, A. & Schulman, H. M. (1980) *Can. J. Biochem.* **58**, 418–426.
32. Huebers, H., Csiba, E., Josephson, B., Huebers, E. & Finch, C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 621–625.