Do monoclonal antibodies Tü15 and Tü67 detect heterogeneity of human transferrin receptor molecules?

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Received 16 July 1984

The possible molecular heterogeneity of human transferrin receptors was analyzed using two murine monoclonal antibodies, Tü15 and Tü67. Both reagents precipitated from lysates of ^{125}I-labeled HL-60 cells a major component of 88 kDa which could be identified as the transferrin receptor by comparison with the proteins detected by monoclonal antibody OKT9. Although sequential immunoprecipitations appeared to demonstrate molecular heterogeneity of transferrin receptors, since the Tü15-reactive species were fully included in the Tü67-positive population, but not vice versa, the possible association of Tü15-reactive molecules with transferrin receptor is also discussed.

Membrane antigen *Transferrin receptor* *Monoclonal antibody* *HL-60 cell* *Receptor heterogeneity* *Sequential immunoprecipitation*

1. INTRODUCTION

Transferrin is the major serum iron-transport protein and plays an essential role in the maintenance of growth of continuous cell lines in culture [1]. It has been suggested that the expression of the receptor for transferrin on the surface of such cells correlates positively with proliferation and activation and is independent of the cell cycle [2–4]. Several monoclonal antibodies (MOABs) have been produced against this molecule [5–7], at least one of which appears to react preferentially with transferrin receptors (TRs) on early erythroid cells [8]. However, molecular heterogeneity of this membrane antigen has never been conclusively demonstrated. We describe two MOABs which have been employed in sequential immunoprecipitation experiments. The results suggest that human TR is molecularly heterogeneous, but the possible association of another molecule recognized by one of these MOABs with TR is also considered.

2. MATERIALS AND METHODS

The human promyelocytic leukemia-derived cell line HL-60 [9] and HP-1, a somatic hybrid between HL-60 and the Burkitt's lymphoma-derived cell line P3HR1 [10], were maintained under standard culture conditions. The MOAB Tü15 (IgM) was produced by fusing P3-NS1/1-Ag4-1 murine myeloma cells [11] with spleen cells from a BALB/c mouse previously immunized with normal human thymocytes, and in the case of Tü67 (IgG), an IL2-driven human T-cell line was the immunogen [12]. All hybrid cell lines were cloned at least twice to assure monoclonality. The MOAB OKT9 is directed against TR molecules [13] and was purchased from Ortho Pharmaceutical. A rabbit antiserum (RαH) was raised against HL-60 cells by a combination of subcutaneous and intravenous injections of intact cells. It was then absorbed extensively on HP-1 cells until only HL-60, but not HP-1 cells were still recognized. Normal rabbit serum served as negative control.

HL-60 cells were labeled with Na^{125}I and lysed as described in [14]. 100 μl of lysate (2×10^6 trichloroacetic acid-precipitable cpm) were incubated for 30 min at 4°C with an equal volume of MOAB-containing culture supernatant or 50 μl of rabbit antiserum after which 40 μl of a 10% *Staphylococcus aureus* strain Cowan I suspension

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00145793/84/$3.00

were added. For immunoprecipitations with MOABs, these bacteria were coated with affinity-purified goat anti-mouse IgG (or IgM) [15]. Finally, the bacterial pellet was washed 5× with phosphate-buffered saline and resuspended in sample buffer [16]. For sequential immunoprecipitations, 500 μl lysate were first precleared 3× with Tü15, Tü67 or rabbit antiserum; the supernatant of the last precipitation was aliquoted and treated again with all reagents used. Normal rabbit serum (NRS) was used as negative control. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was done on 7% slab gels under reducing conditions [16] and molecular mass markers (New England Nuclear) were employed. All gels were autoradiographed on Fuji RX films.

3. RESULTS

The MOABs Tü15 and Tü67 as well as OKT9 reacted with all HL-60 cells; however, when these cells were induced to differentiate towards macrophages or granulocytes, all 3 antibodies ceased to react with the non-dividing, differentiated cells [17] (not shown). Since TRs disappear from the cell surface of non-proliferating cells, it was possible that this protein was the target of the MOABs. A comparison of the material precipitated by Tü67 and OKT9 showed that the molecular species recognized by the two MOABs were indistinguishable (fig.1A). In each case, a major band of 88000 Da, the TR molecule [6,7], was precipitated together with minor bands of M_r 180000, 155000 and 145000, presumably dimers of the receptor and receptor–transferrin complexes. An additional band at 76000 Da might be transferrin itself. Sequential immunoprecipitation using Tü67 as first reagent proved that Tü67 and OKT9 detected identical molecules (not shown), since the Tü67-depleted lysate did not contain OKT9-reactive molecules any longer. By comparison with Tü67, Tü15 also recognized human TRs, but much less cpm could be observed in the 88000 Da band (fig.1B). When Tü15 and Tü67 were added simultaneously, the amount of radioactivity in the TR band resembled that seen with Tü67 alone. The RαH serum directed against HL-60 cells was absorbed with HP-1 cells, because these had been found to lack a variety of cell surface molecules

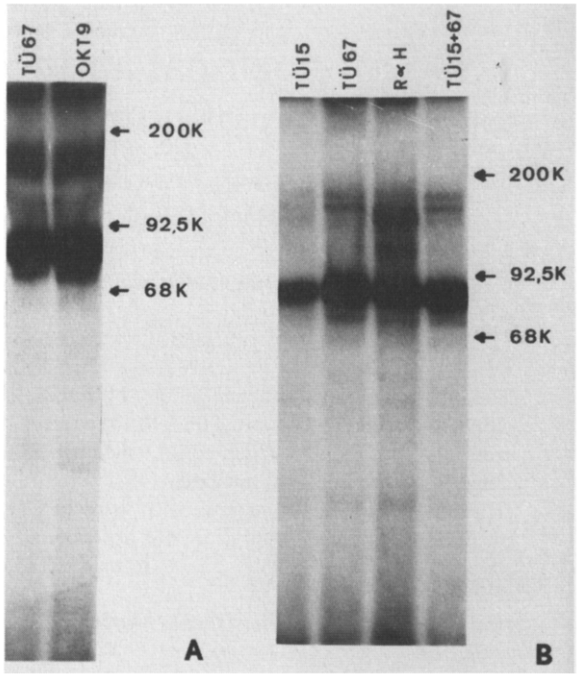

Fig.1. Autoradiograph of 7% SDS gels of immunoprecipitates from ^{125}I-labeled HL-60 cells under reducing conditions. Positions of molecular mass standards are indicated (200 kDa, myosin; 92.5 kDa, phosphorylase *b*; 68 kDa, bovine serum albumin; 43 kDa, ovalbumin).

specific for myeloid cells [10]. The resulting antiserum precipitated several molecules, but by far the major band again appeared to be TR (fig.1B).

Sequential immunoprecipitations were used to analyze the relationship of the molecules detected by Tü15, Tü67 and RαH serum (figs 2,3). Three cycles of precipitation with Tü15 were sufficient to completely eliminate the molecules recognized by this MOAB (fig.2c), but both Tü67 and RαH were still able to detect TR proteins (fig.2d,e). However, when Tü67 was used as first reagent, not only the Tü67-positive molecules were removed as expected (fig.2j), but also the proteins recognized by Tü15 (fig.2i) and those bands precipitated by RαH (fig.2k) which correlate in molecular mass with the molecules detected by Tü67 (see fig.1B). When Tü15 and Tü67 were employed simultaneously for the preprecipitation (fig.3g–l), the result was no different from that obtained with Tü67 alone. The RαH serum also recognized TR, since no Tü15- or

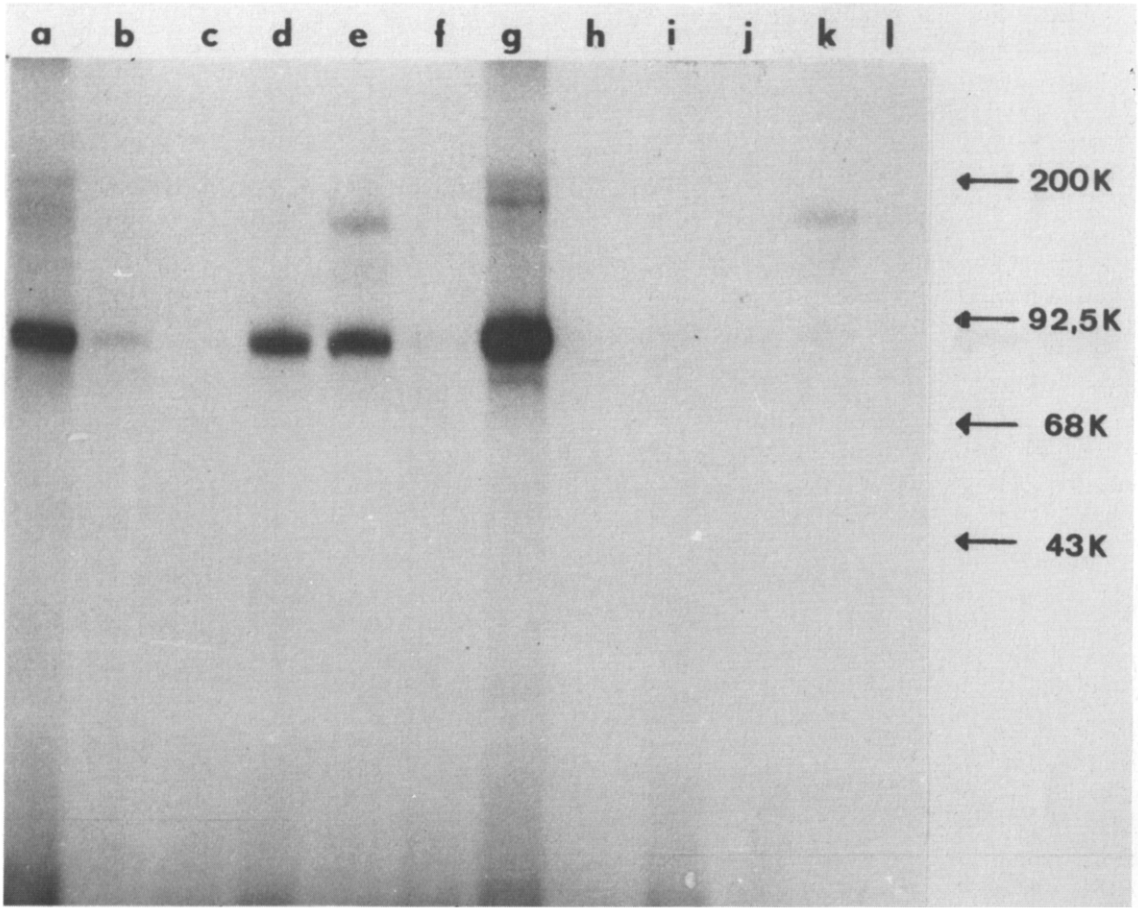

Fig.2. Sequential immunoprecipitation of molecules recognized by Tü15, Tü67 and RαH from ^{125}I-labeled HL-60 cell lysate. Autoradiograph of 7% SDS gels. Lanes: a–c, first, second and third precipitation respectively with Tü15; d, Tü15-depleted cell lysate precipitated with Tü67; e, exactly as line d, but precipitation with RαH; f, NRS control. Lanes: g,h,j, first, second and third precipitation respectively with Tü67; i, Tü67-depleted cell lysate precipitated with Tü15; k, exactly as i but precipitation with RαH; l, NRS control. All samples were reduced.

Tü67-reactive proteins remained in the lysate after depletion with the xenoantiserum (fig.3a–f).

4. DISCUSSION

The results presented here show that it is easily possible to produce a conventional antiserum with preferential activity against human TR. This serum will be useful in in vitro translation studies, since MOABs often fail to recognize the proteins synthesized in these systems [18]. However, what appears more interesting at this point is the molecular complexity of human TR detected by MOABs. Differential reactivity of certain MOABs directed against TR has been shown before using various bone marrow-derived cells [8], but two types of TR on a given cell have so far not been described. The sequential immunoprecipitation experiments show that the receptor population detected by Tü15 is only part of that recognized by Tü67 or RαH serum. Since Tü15 and Tü67 each react with 100% of uninduced HL-60 cells, the differential activity of Tü15 to the receptor molecules cannot be due to

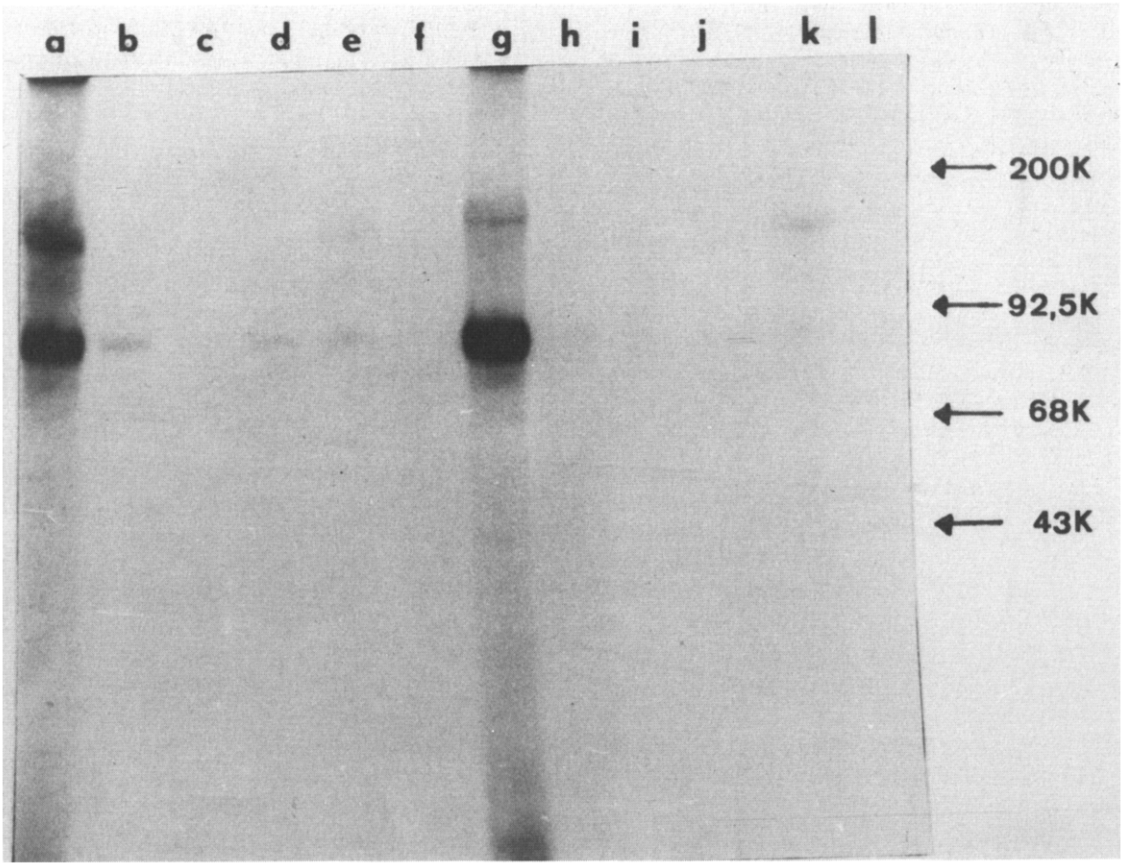

Fig.3. Autoradiograph of 7% SDS gel as in fig.2. Lanes: a,b,e, first, second and third precipitation with RαH serum; c, RαH-depleted cell lysate precipitated with Tü15; d, as lane c, but precipitation with Tü67; f, NRS control. Lanes: g,h, first and second precipitation with Tü15 + Tü67; i, Tü15 + Tü67-depleted cell lysate precipitated with Tü15; j, as lane i, but precipitation with Tü67; k, as lane i, but precipitation with RαH; l, NRS control.

reactivity of this MOAB with only a subpopulation of cells. It is also highly unlikely that weak affinity of Tü15 is responsible for the restricted activity observed, since after 3 cycles of preprecipitation, no Tü15-positive molecules could be detected, although TR proteins could be easily demonstrated in the depleted lysate by using Tü67 or RαH serum. Therefore, the simplest conclusion would be that Tü15 and Tü67 distinguish two types of human TR. An alternative possibility might be that Tü15 detects a molecule of similar size to TR and complexed to the latter, but distinct. This may lend the TR functional heterogeneity with regard to cell growth and differentiation.

ACKNOWLEDGEMENTS

We thank Ms J. Heinig for her skilled help. This work was in part supported by the Deutsche Forschungsgemeinschaft through SFB 120.

REFERENCES

[1] Barnes, D. and Sato, G. (1980) Cell 22, 649–655.
[2] Larrick, J.W. and Cresswell, P. (1979) J. Supramol. Struct. 11, 579–586.
[3] Neckers, L.M. and Cossman, J. (1983) Proc. Natl. Acad. Sci. USA 80, 3494–3498.

[4] Newman, R., Schneider, C., Sutherland, R., Vodinelich, L. and Greaves, M. (1982) Trends Biochem. Sci., 397–400.
[5] Omary, M.B., Trowbridge, I.S. and Minowada, J. (1980) Nature 286, 888–891.
[6] Sutherland, R., Delia, D., Schneider, C., Newman, R., Kemshead, J. and Greaves, M.F. (1981) Proc. Natl. Acad. Sci. USA 78, 4515–4519.
[7] Trowbridge, I.S. and Lopez, F. (1982) Proc. Natl. Acad. Sci. USA 79, 1175–1179.
[8] Lebman, D., Trucco, M., Bottere, L., Lange, B., Pessano, S. and Rovera, G. (1982) Blood 59, 671–678.
[9] Collins, S.J., Gallo, R.C. and Gallagher, R.E. (1977) Nature 260, 347–349.
[10] Koeffler, H.P., Sparkes, R.S., Billing, R. and Klein, G. (1981) Blood 58, 1159–1163.
[11] Köhler, G. and Milstein, C. (1976) Eur. J. Immunol. 6, 511–519.
[12] Shi, L.G., Schneider, E.M., Ziegler, A., Wernet, P. and Pawelec, G. (1984) in: Leucocyte Typing (Bernard, A. et al. eds) Abstract, pp.734–735, Springer, Berlin.
[13] Reinherz, E.L., Kung, P.C., Goldstein, G., Levey, R.H. and Schlossman, S.F. (1980) Proc. Natl. Acad. Sci. USA 77, 1588–1592.
[14] Ziegler, A. and Pink, R. (1976) J. Biol. Chem. 251, 5391–5396.
[15] Ziegler, A., Uchańska-Ziegler, B., Zeuthen, J. and Wernet, P. (1982) Somat. Cell Genet. 8, 775–789.
[16] Ziegler, A. and Milstein, C. (1979) Nature 279, 243–244.
[17] Uchańska-Ziegler, B., Wernet, P. and Ziegler, A. (1983) in: Modern Trends in Human Leukemia V (Neth, R. et al. eds) Haematology and Blood Transfusion 28, pp.386–388, Springer, Berlin, Heidelberg.
[18] Algranati, I.D., Milstein, C. and Ziegler, A. (1980) Eur. J. Biochem. 103, 197–207.