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^{125}I-ANTI-INSULIN RECEPTOR FAB IS INTERNALIZED BY CULTURED HUMAN LYMPHOCYTES

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Summary: When ^{125}I-anti-insulin receptor Fab components are prepared from serum IgG, this univalent material binds to the surface of cultured human lymphocytes and is subsequently internalized by the cell in an identical fashion to the divalent IgG and to ^{125}I-insulin itself.

Spontaneous autoantibodies against the insulin receptor occur in a rare group of patients who exhibit hypoglycemia, hyperglycemia, or both. When these anti-insulin receptor antibodies (anti-R-Ab) are partially purified and iodinated they bind to insulin receptors of cultural human lymphocytes in a competitive fashion (1). Further, these divalent immunoglobulins are internalized by cultured human lymphocytes in a manner similar to insulin itself (2).

The monovalent Fab component of the anti-R-Ab also binds to the insulin receptor but, unlike the divalent molecule which exhibits insulin-like biologic effects, the Fab component is biologically inactive (3).

Since monovalent IgG (4) and IgE (5) molecules are not usually internalized by cells, we wished to determine whether the monovalent anti-R-Ab Fab component behaves like insulin and the divalent anti-R-Ab with respect to internalization.

MATERIALS AND METHODS

Cell Culture: Cultured human lymphocytes of the IM-9 cell line were used for all experiments. Cells were grown and used as previously described (2).

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Preparation of Antireceptor ^{125}I-Fab: Antireceptor IgG was purified from the serum of patient B-2 by acid elution from protein-A Sepharose and monovalent anti-receptor Fab prepared and iodinated as previously described (6). The ^{125}I-Fab is then further purified by cytoadsorption to cultured human lymphocytes. Binding and internalization of the final ^{125}I-Fab preparation to IM-9 lymphocytes is completely inhibited by an excess of unlabeled insulin.

Incubation and Morphologic Evaluation: Incubation buffer and conditions were exactly as previously described for antireceptor IgG (2). Following appropriate incubation with ^{125}I-Fab at the temperature specified, cells were fixed, processed for electron microscopy and prepared for autoradiography (2).

RESULTS AND DISCUSSION

When ^{125}I-anti R-Fab is incubated with cultured human lymphocytes of the IM-9 line, the ligand, at early times of incubation, localizes primarily to the cell surface as determined by quantitative EM autoradiography (Figure 1). When the incubation is continued for 60 to 120 minutes at 37°C, the labeled material is internalized to a small but consistent extent (Figure 1 and Table I).

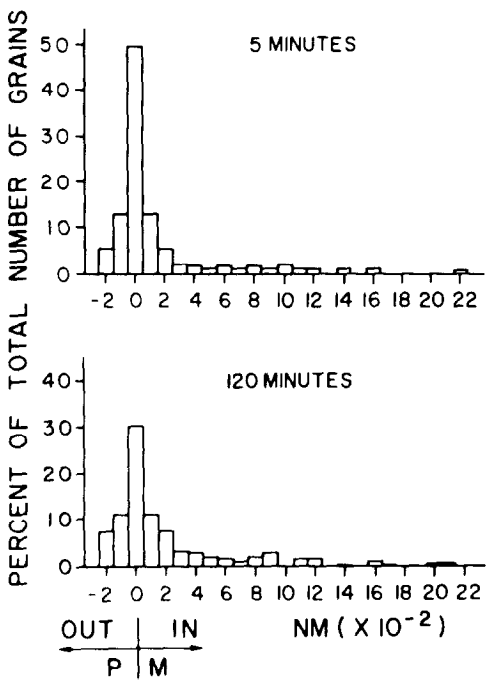

Figure 1. Grain Distribution Histogram ^{125}I-anti-receptor Fab. Approximately 150 developed autoradiographic grains are photographed for each time point shown here and in Table I. The percentage of total grains (vertical axis) is plotted as a function of the distance of the grain center from the 0 point or plasma membrane (horizontal axis). The spreading of grains to the right and decrease over the plasma membrane represents internalization of the ^{125}I-labeled ligand. For further details see reference 2.

Table I = Percentage of labeled material internalized by IM-9 lymphocytes

	^{125}I-Fab		^{125}I-AIRA**	^{125}I-insulin+
	Expt.-1	Expt.-2		
5 minutes*	0	0	0	0
30 minutes	1.8	5.0	1.2	2.1
60 minutes	6.2	9.4	-	3.2
120 minutes	18.0	18.8	10.4	10.0
180 minutes	-	-	16.8	17.3

* = To calculate % translocation, the 5 minute time point was used as control. For each time point the % translocation is, therefore, the percent gains beyond 250 nm minus the percentage of gains beyond 250 nm for the 5 minute incubation

** = Results reported in this column are derived from reference 2 and are the mean of two separate experiments

+ - Results derived from references 7,8

The degree of internalization of the ^{125}I-anti-R-Fab is similar to that previously observed for the divalent ^{125}I-anti-R-Ab and for ^{125}I-insulin itself.

This study demonstrates directly that ^{125}I-anti-R Fab is internalized by cultured human lymphocytes in a manner analogous to the internalization of the divalent ^{125}I-anti-R-Ab (2) and to ^{125}I-insulin itself (7,8).

Though in special cases Fab components of IgG that bind to receptors other than hormone receptors may be internalized (9), this is not usually the case (4). Further, Fab components of IgE are not internalized under similar conditions (5).

Thus, in the case of polypeptide hormone receptors, univalent ligands that bind to the receptors such as the hormone per se and the biologically inactive Fab component are processed in a similar fashion. This is consistent with the notion that internalization provides a mechanism for clearing a ligand from the cell surface for delivery to an intracellular degradation site and that this process is likely unrelated to the mechanism that transduces a biologic signal from the hormone receptor complex.

ACKNOWLEDGMENT

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REFERENCES

1. Jarrett, D.B., Roth, J., Kahn, C.R., and Flier, J.S. (1976) Proc. Nat. Acad. Sci., U.S.A. 73:4115-4119.

2. Carpentier, J.-L., Van Obberghen, E., Gorden, P., and Orci, L. (1981) Exp. Cell Res. 134:81-92.

3. Kahn, C.R., Baird, K.L., Jarrett, D.B., and Flier, J.S. (1978) Proc. Nat. Acad. Sci., U.S.A. 75:4209-4213.

4. Loor, F. (1974) J. Cell Biol. 63:12-23.

5. Isersky, C., Rivera, J., Segal, D.M., and Triche, T. (1983) J. of Immunology (in press).

6. Kasuga, M., Van Obberghen, E., Yamada, K.M., and Harrison, L.C. (1981) Diabetes 30:354-357.

7. Gorden, P., Carpentier, J.-L., Van Obberghen, E., Barazzone, P., Roth, J., and Orci, L. (1979) J. Cell Sci. 39:77-88.

8. Carpentier, J.-L., Gorden, P., Amherdt, M., Van Obberghen, E., Kahn, R.C. and Orci, L. (1978) J. Clin. Invest. 61:1057-1070.

9. Antoine, J.C., Avrameas, S., Gonatas, N.K., Stieber, A., and Gonatas, J.O. (1974) J. Cell Biol. 63:12-23.