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Cell surface changes during adipocyte differentiation *in vitro*

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Introduction.

Not least among the notably important advances in knowledge concerning the origin and development of white adipose tissue has been the recognition of the adipocyte precursor and the demonstration that such cells can be isolated from mature tissue and be induced to differentiate to adipocytes *in vitro.* The interest generated in the nature, origin, proliferation and differentiation of the adipocyte precursor has lead to studies on cells of human (Van *et al.,* 1976), rat (Björntorp *et al.,* 1978 ; Roncari *et al.,* 1979), mouse (Négrel *et al.,* 1978), bovine (Plaas and Cryer, 1980 ; Cryer *et al.,* 1984), ovine (Broad and Ham, 1983) and avian origins (Cryer J. and Cryer A., unpublished observations). Additionally the 3T3-L_1 mouse embryo fibroblast clone and other related clones have also been used extensively as a model of adipocyte differentiation *in vitro* (see for example Green, 1979 ; and Ailhaud, 1982, for review).

The patterns of enzymatic differentiation displayed by precursor cells isolated from mature and from embryonic sources are now well characterized and are compatible with the adipocyte nature of the cells following their differentiation in culture (Green, 1979 ; Cryer, 1980, 1982). Although certain information has become available (Sidhu, 1979 ; Reed *et al.,* 1980 ; Plaas *et al.,* 1981) with regard to the 3T3-L_1 cell clone, relatively little is known of the cell surface changes that characterize adipocyte differentiation in precursors of mature tissue origins (Cryer *et al.,* 1984).

Because immunological assay methods have proved so useful in the study of cell surfaces in many other differentiating cell systems (Williams *et al.,* 1977 ; Trisler *et al.,* 1979) the possibilities for the use of an immunologically-based technique for studying adipocyte differentiation have been pursued.

The production and characterization of adipocyte specific antisera.

Rabbit anti-adipocyte sera have been prepared using adipocytes and adipocyte plasma membrane fractions derived from mouse (Plaas *et al.,* 1981), bovine (Cryer *et al.,* 1984), rat (Lee S. R. and Cryer A., submitted for publication)

and chicken (Cryer J. and Cryer A., unpublished observations) sources as the immunogen. Each antiserum was characterized with regard to the concentration required for optimal binding to adipocyte and plasma membranes of the appropriate source and with regard to the concentration range over which binding was related to cell volumes or amount (μg) protein of plasma membrane used. The procedure for quantitating the binding of adipocyte specific antibodies to adipocytes may be described as an indirect-labelled-second-antibody cellular immunoassay and is described in figure 1.

Using the assay as described under optimal conditions the reactivity of antisera with adipocytes derived from different species has been tested (table 1). Antisera raised to bovine adipocytes showed a high level of reactivity toward bovine material but a low level of reactivity toward rat and chicken material. Similarly the chicken adipocyte antiserum reacted strongly with chicken material but weakly with bovine or rat material. By contrast to the low level of reactivity shown by antisera raised against rat adipocytes with bovine or chicken material considerable levels of cross-reactivity were evident when rat and mouse material were compared. This interrodent cross reactivity was also obvious when the

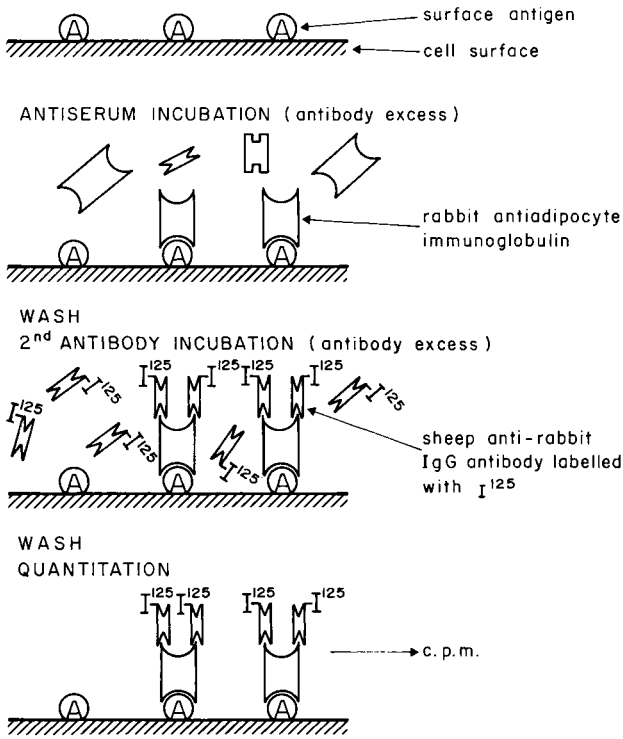

FIG. 1. — *Procedure for the quantitation of antiadipocyte antibody binding to cell surfaces or plasma membrane preparations.*

The practical details of the procedure and the preparation of the reagents used have been described extensively in Plaas *et al.* (1981) and Cryer *et al.* (1984).

TABLE 1

Test of crossreactivity of antisera raised and tested against adipocytes from a number of species.

Species from which adipocytes for immunization was prepared	Origin of adipocyte material used in indirect-labelled-second antibody immunoassay			
	Bovine	Rat	Chicken	Mouse
Bovine	100 %	24 %	26 %	—
Rat	32 %	100 %	5 %	85 %
Chicken	15 %	5 %	100 %	—
Mouse	—	59 %	—	100 %

Optimal concentrations of the antisera raised in rabbits to adipocytes from the species listed were used to test their reactivities against adipocyte plasma membranes prepared from adipocytes of the species shown.

antimouse serum was studied with regard to its reactivity against rat and mouse adipocyte surfaces.

Although the adipocyte-specific antisera will react with certain other cell types, appropriate absorption procedures have been employed to remove such reactivities without comprising the reactivity towards adipocytes (see Plaas *et al.*, 1981 ; Cryer *et al.*, 1984).

Investigation of the reactive antigens present on adipocyte surfaces.

The nature of the components present in adipocyte plasma membranes that react with the antiadipocyte sera has also been investigated. Thus plasma membranes prepared by the method of Belsham *et al.* (1980) from bovine, chicken and rat adipocytes have been iodinated using a lactoperoxidase method and the labelled polypeptides, analysed by sodium dodecyl sulphate gradient polyacrylamide gel electrophoresis (Laemmli, 1970 ; Siemankowski and Dreizen, 1978) (Tume R. K., Lee S. R. and Cryer A., submitted for publication).

The immunoprecipitates produced when such solubilized labelled membrane preparations were incubated with optimal concentrations of antisera were also analysed by gel electrophoresis. Specific labelled components were only precipitated by sera raised against the same species of adipocytes from which the experimental plasma membrane were derived. Optimally between 1.5 and 3 % of the solubilized radioactivity was precipitated. When cross-species immunoprecipitations were attempted no specifically precipitated components were detected, which was also the case when non-immune sera were used.

The major components precipitated from the plasma membrane preparations that were either prelabelled with ^{125}I or which remained unlabelled, had molecular weights of 120 000, and 92 000 and 59 000 in the case of the rat, 87 000 in the case of the bovine and 56 000, 47 000 and 37 000 in the case of the chicken.

The cell surface orientation of the antigens described above has been confirmed by a combination of cellular fractionation studies and electronmicrographic evidence.

Changes in precursor cell reactivity during differentiation *in vitro*.

When the indirect labelled-second-antibody cellular immunoassay, using antisera against adipocytes of the appropriate species, was used to follow the changes in immunoreactivity exhibited by monolayers of 3T3-L_1, bovine precursor and rat precursor cells at various stages of their postconfluent culture development the patterns of change were as shown in Table 2. With precursor cells derived from mature tissue the time course of the change in immunoreactivity was similar in the bovine and rat cases with a relatively progressive assent to maximal reactivity at approximately 10 days. Although the progression with 3T3-L_1 cells cannot be compared directly with the other two because of the differences in culture conditions the pattern in the case of these embryo-derived cells seems to be defined by two phases. Thus 30 % of the maximal reactivity arises within 2-4 days of the cells reaching confluency and this is followed by a sudden increase to maximal levels at day 5 post confluence.

A comparison of these cell surface changes with changes in cellular lipoprotein lipase activity, which may be considered an enzymic marker for the adipocyte phenotype, are shown in the case of the bovine precursors in figure 2. It is clear that in this study at least the changes in cell surface immunoreactivity precede the emergence of substantial lipoprotein lipase activity and that neither are dependent upon the presence of insulin in the medium. Furthermore we have found that for bovine, rat and chicken precursors the emergence of lipoprotein lipase activity and the accumulation of intracellular lipid during the development of these cells post-confluence is not dependent on the presence of insulin supplements in the medium as they seem to be in the case of 3T3L_1 cells.

The next stage of this work which must now be attempted is to determine the functional corellates of the adipocyte antigens described and to relate the

TABLE 2

Comparison of adipocyte-specific immunoreactivity displayed by precursor cells during their differentiation in vitro.

Days post confluence	% maximum immunoreactivity		
	3T3L_1	Bovine precursors	Rat precursors
0	3	24	24
1	16	—	—
2	33	34	—
3	27	—	56
4	37	66	—
5	100	—	48
6	97	71	—
7	90	—	86
10	—	95	80
10-30	—	100	100

The data has been calculated from that given for 3T3L_1 cells by Plaas *et al.* (1981), bovine precursor cells by Cryer *et al.* (1984) and rat precursor cells A. Cryer and K. Cakebread (unpublished observations). The 3T3-L_1 cells were induced to differentiate using the procedure described by Rubin *et al.* (1978).

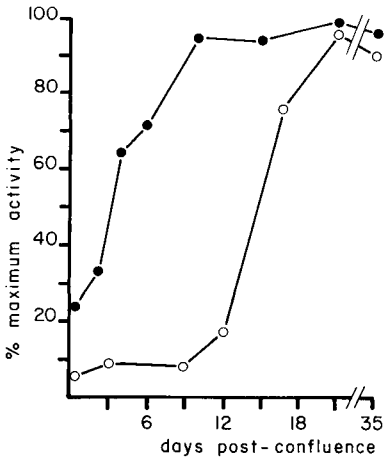

FIG. 2. — *Comparison of changes in surface immunoreactivity and total cellular lipoprotein lipase activities during differentiation of bovine adipocyte precursor cells in vitro.*

Bovine adipocyte precursor cells were prepared and cultured as described in Plaas and Cryer (1980). Adipocyte-specific immunoreactivity (●——●) was determined as described in the text on monolayers of cells grown in 25 cm^2 flasks. Similar cells grown in 75 cm^2 flasks provided material for the preparation of acetone-ether powders for the measurement of lipoprotein lipase activity (○——○) as described in Cryer and Jones (1978).

changes in their cell surface concentration with the progression of adipocyte differentiation.

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Résumé. *Modifications des surfaces cellulaires au cours de la différenciation* in vitro *des adipocytes.*

Des antisérums spécifiques de cellules et d'espèces réagissant fortement avec la surface externe de la membrane plasmique des adipocytes provenant de rats, souris, bovins et poulets ont été préparés. Les conditions optimales de réaction de ces antisérums avec des adipocytes, des préparations de membranes plasmiques et des précurseurs d'adipocytes à différents stades de leur différenciation *in vitro* ont été déterminées à l'aide d'un dosage radioimmunologique cellulaire à marquage indirect utilisant un second anticorps. Dans tous les cas, le faible niveau d'immunoréaction observé avec des suspensions cellulaires fraîchement préparées s'accroît considérablement au cours de la période de post-confluence pendant laquelle les précurseurs se différencient. Les modifications de la surface cellulaire tendent à précéder celles des activités enzymatiques, qui sont également caractéristiques de l'évolution de ces cellules vers l'état d'adipocyte. Les poids moléculaires des composants présents dans les membranes plasmiques des adipocytes différenciés et qui constituent les « marqueurs d'adipocytes » ont été déterminés dans le cas du rat, du poulet et des bovins ; il a été montré qu'ils sont caractéristiques de chaque espèce.

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