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Common antigenicity between a human thymic epithelial cell product and a thymus-dependent serum factor¹

E.F. Potworowski, Danuta Kowalczyk, V.V. Micusan and T. Seemayer

Immunology Research Center, Armand-Frappier Institute, P.O. Box 100, Laval (Québec, Canada H7N 4Z3), and Department of Pathology, Montreal Children's Hospital, Montreal (Canada), 30 November 1981

Summary. Specific immunofluorescence of human thymic epithelial cytoplasm was obtained with antibodies to supernatant of thymic epithelial cultures, and with anti-prealbumin antibodies. These antibodies also reacted with normal serum but not with serum from Di George patients. The data indicates that thymic epithelium and a component of the prealbumin fraction of normal serum share a common antigen believed to be thymic hormone.

Thymic hormone activity has been described in a number of thymic extracts, in the supernatant of cultured thymic epithelial cells (thymic epithelial supernatant or TES)^{2,3}, in the prealbumin fraction of serum⁴ and in several synthetic polypeptides^{5,6}. Recent efforts by various groups have shown that antibodies to the synthetic polypeptide FTS (facteur thymique sérique) bind thymic epithelial cells^{7,8}. In this work, we present evidence in support of a thymic epithelial origin of the factor bound to prealbumin in normal individuals. Antibodies to prealbumin were obtained from Behring diagnostic. The preparation of TES has previously been described⁹. Antibodies to TES were prepared in rabbits according to a standard protocol¹⁰. Normal human serum was obtained from healthy volunteers and sera from Di George patients, devoid of a functional thymus, were a gift of Drs C. Griscelli and E. Reece.

Anti-TES serum applied to frozen sections of fresh human thymus with a fluorescein conjugated goat anti-rabbit serum stained strongly and exclusively the cytoplasm of reticuloepithelial cells. While positively staining individual epithelial cells were found both in the medulla and in the cortex, an accumulation of these cells were found to line the outer cortical layer, immediately adjacent to the connective tissue capsule or the interlobular septum (fig). Thymic epithelial cells grown in culture were also examined in the same way. While not all cells were found to be positively stained, those that were displayed a spotty cytoplasmic pattern often extending into the cytoplasmic processes. Cultured human fibroblasts treated in the same fashion did not display any fluorescence.

Anti-prealbumin serum was used without any adsorption in the same way as the anti-TES serum. Whether tested on frozen sections of human thymus, on cultured thymic epithelium, or on cultured human fibroblasts, the reaction was in all cases identical to that obtained with the anti-TES serum.

The presence of TES antigen in human serum was tested by immunofluorescence on thymic sections using anti-TES serum after various absorptions. Anti-TES serum was absorbed for 2 h at 37 °C with normal human serum, with Di George serum, with TES or with phosphate buffered saline (PBS) in various ratios and applied to serial frozen sections of human thymus, using in all cases a conjugate dilution of 1:8. The results (table 1) show that normal human serum and TES abolished the reaction of anti-TES with thymic epithelial cells, while Di George serum and PBS were unable to do so.

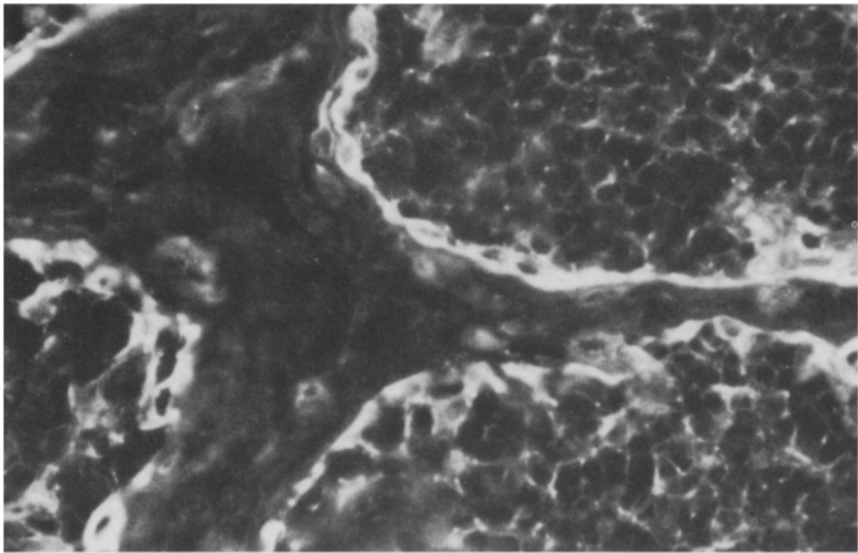
Similarly anti-prealbumin serum was absorbed with normal human serum, with Di George serum, with TES or with PBS and applied to serial frozen sections of human thymus. Here again (table 2), normal human serum and TES completely absorbed out the antibodies in anti-prealbumin directed against thymic epithelial cells, while Di George serum and PBS had no such effect.

In order to determine whether anti-prealbumin serum had a single antibody directed against an antigen shared by

Table 1. Intensity of immunofluorescence staining of epithelial cells in thymic sections treated with an antiserum to thymic epithelial supernatant^a (TES), after various absorptions

Anti-TES serum/absorbant ratio	Absorbants Normal human serum	Di George serum	TES	PBS
3:1	++(+)/++	++(+)	(+)	++(+)/++
2:1	++	++(+)	-	+++
1:1	-	++	-	+++

^a Anti-TES prepared in rabbits was used with goat anti-rabbit fluorescein conjugate. ^b Fluorescence intensity was evaluated in arbitrary units ranging from - (negative) to ++++ (strongly positive).



Frozen section of human thymus treated with rabbit anti-TES and fluorescein-conjugated goat anti-rabbit serum. Note the accumulation of fluorescing reticuloepithelial cells lining the interlobular septum (approximately $\times 750$).

Table 2. Intensity of immunofluorescence staining of epithelial cells in thymic sections treated with anti-prealbumin serum^a, after various absorptions

Anti-prealbumin serum/absorbant ratio	Absorbants Normal human serum	Di George serum	TES	PBS
1:2	+ + / + + + ^b	+ + + / + + + +	+ + / + + +	+ + (+) / + + +
1:4	+	+ + (+)	+	+ + (+)
1:6	-	+ +	-	+ +

^a Anti-prealbumin serum prepared in rabbits was used with a goat anti-rabbit fluorescein conjugate. ^b Fluorescence intensity was evaluated in arbitrary units ranging from - (negative) to + + + + (strongly positive).

prealbumin and TES, or whether it had 2 distinct antibody specificities, immunodiffusion tests were performed between unabsorbed anti-prealbumin serum and anti-prealbumin serum absorbed with TES 1:6 on the one hand, and normal and Di George serum on the other hand. In both cases there were precipitation lines displaying an identity pattern between normal human serum and Di George serum. Since the anti-prealbumin serum/TES ratio used in the absorption was sufficient to block the antibodies to epithelial cells in the immunofluorescence test, but did not in any way alter the precipitin reaction between anti-prealbumin and normal or Di George serum, it is safe to conclude that these 2 types of reaction were mediated by 2 distinct antibodies present in the anti-prealbumin serum: the 1st, directed against TES, which formed a non-precipitating complex and the 2nd directed against pre-albumin which seems to be identical in normal and Di George serum.

We have shown that thymic secretions (TES) and the

prealbumin fraction of normal human serum share a common antigenicity, and that this antigenicity is absent from the sera of Di George patients. Thymic hormone activity has been found both in the serum prealbumin fraction^{4,11} and in the thymic secretion^{9,12,13}. Furthermore, in addition to sharing biological activity¹⁴, thymic secretion and prealbumin associated factors also share a common antigenicity and localize in the same medullary and outer cortical epithelial cells as those where thymosin α 1 and thymosin β 3 were recently described¹⁵. Taken together these data lend support to the idea that thymic epithelial cell secretions are carried in the circulation by prealbumin.

Thus human prealbumin when isolated from healthy donors for the preparation of anti-prealbumin antibodies also contains thymic factor against which antibodies are made. The latter, however, are non-precipitating and therefore distinct from the precipitating prealbumin/anti-prealbumin system detectable equally well in normal and in Di George sera.

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