Instance of metabolic stimulation by antibody

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To quote Haurowitz [1], "the notion of immunity involves protection against a harmful agent", and antibodies, as the mediators of the protection, are generally seen in a similar context. That antibodies might on occasion stimulate a biological process, rather than destroy or inhibit it, has been suggested periodically, and more frequently of late. There would seem to be no better example, however, of biological stimulation by an antibody than the phenomenon of the long-acting thyroid stimulator of Graves' disease.

Although for years the hyperthyroidism of Graves' disease was considered to reflect an inordinate effect of the normal thyroid-stimulating hormone, thyrotropin, this was always in dispute [2]. During the last 12 years it has become apparent that another agent, first recognised by Adams and Purves [3] and now known as the long-acting thyroid stimulator, is the cause of the intense hyperfunction found in that disorder; since 1964 it has increasingly been accepted that this stimulator is a γ-globulin, an IgG.

Is the long-acting thyroid stimulator an antibody?

Meek et al. [4] first obtained an electrophoretically pure preparation of a long-acting thyroid-stimulating γ-globulin, but Kriss et al. [5] should be credited with establishing beyond reasonable doubt that the long-acting thyroid stimulator, when it occurs, is an integral component of the IgG moiety of serum protein. The techniques used to prove this included paper electrophoresis, immunoelectrophoresis, double diffusion in agar, analytical ultracentrifugation, sucrose density-gradient separation, and inactivation by anti-IgG antibody [5]. More recently they reported [6] that anti-k serum inhibited 40% of the biological activity of a preparation of the long-acting thyroid stimulator and anti-λ serum inhibited the remaining 60%; apart from these findings further proving that the stimulator is indeed an immunoglobulin, they were taken to indicate that it is a polyclonal product,

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rather than monoclonal which would be expected to contain only λ or k form of L chain. (This interpretation, which meets expectations derived from other considerations [2], is of course dependent upon the monospecificity of the anti-λ and anti-k sera used, and this evidence has not yet been presented.)

While proof that the long-acting thyroid stimulator is a member of the immunoglobulin class of proteins seems conclusive, that in itself does not prove it is an antibody. Recently corroborative evidence has appeared with reports [7–9] that an antibody can be produced by immunizing rabbits with extracts of human thyroid and the antibody acts as a thyroid stimulator in the assay animal, i.e. the mouse [10]. Attempts are being made in several laboratories to isolate the antigen corresponding to this antibody for this will be required before there is final acceptance of the long-acting thyroid stimulator as an antibody; however, the evidence currently available is strongly in support of the concept.

The long-acting thyroid stimulator as a metabolic stimulant

Intrinsic to the claims that the long-acting thyroid stimulator is both an antibody to a thyroid component and the cause of hyperthyroidism in Graves' disease is the implication that it stimulates the metabolism and function of the thyroid gland. There is no doubt that this is the case, although the precise mode of action remains uncertain. Stimulation of radioiodine metabolism of the thyroid – which is the basis of the bioassay of the stimulator [10] – has been proven in many ways [2] and is most probably mediated by 3′,5′-adenosine monophosphate (cyclic AMP) [11]. Early it was shown *in vivo* with mice that the morphological criteria of thyroid stimulation were met [12]; repeated injections of serum containing the long-acting thyroid stimulator resulted not only in an increase in the rate of release from the thyroid (in the form of thyroid hormone [13]) of previously injected radioiodine and in the rate of uptake of ^{131}I by the gland, but also caused an elongation of the thyroid cell and resorption of acinar colloid. By *in vitro* studies it was shown that preparations of thyroid-stimulating IgG enhanced the rates of glucose oxidation and ^{32}P incorporation into phospholipid in sheep [14] and dog [15] thyroid slices. Most recently P. R. Adiga (unpublished data) has observed striking increases on addition of thyroid-stimulating IgG, in the rates of incorporation of ^{3}H-labelled leucine into protein and ^{3}H-labelled uridine into RNA, using slices of porcine thyroid; increases were observed at the earliest time interval studied, i.e. 1 hour after the addition of radioactive precursor.

The manner in which an IgG can lead to metabolic stimulation of the thyroid, both *in vivo* and *in vitro* is clearly a subject leading to much speculation, particularly since its actions are qualitatively indistinguishable from those of thyrotropin, apart from the speed at which stimulation is initiated (the action of thyrotropin, both *in vitro* and *in vivo*, is more rapid and, at

least *in vivo*, more evanescent). Perhaps the easiest way to conceive of stimulation taking place is to see the IgG suppressing a tonic inhibitor of thyroid function, even though such an inhibitor is not yet recognized. As noted above, cyclic AMP possibly mediates the action of the long-acting thyroid stimulator (and thyrotropin [15]) in its effects on metabolism of radioiodine; furthermore, the influence of thyrotropin on thyroid gland oxidation of glucose and ^{32}P incorporation into phospholipid may be similarly mediated [15]. P. R. ADIGA and P. V. N. MURTHY have evidence (unpublished) that the cyclic nucleotide can stimulate incorporation of precursor into protein and nucleic acids of porcine thyroid slices in a manner analogous to the effects of the long-acting thyroid stimulator. Consequently it may be that all aspects of metabolic stimulation of the thyroid gland brought about by this immunoglobulin are mediated by one action involving cyclic AMP, but much more evidence will be required to prove it. Certainly there is some attraction to the hypothesis since adenyl cyclase, which forms cyclic AMP from ATP, is apparently bound to the peripheral membrane of the thyroid cell, as it is to the cells of other organs, and it is easier to conceive of an immunoglobulin's acting at the surface of a cell, rather than within.

A non-thyroid action of the long-acting thyroid stimulator

Thyrotropin is known to influence the metabolism of adipose tissue (although this is a teleologically puzzling phenomenon) and I. R. HART has investigated the effects of IgG, prepared from serum containing the long-acting thyroid stimulator, on the *in vitro* metabolism of epididymal fat from guinea pigs. Amongst other findings, he observed (unpublished) a marked stimulation (7–10 fold) of lipolysis, as judged by the release of unesterified fatty acids, upon addition of 1.75 mg to 7 mg of the thyroid-stimulating IgG, while 3.5 mg of IgG from normal human serum had no effect. In this instance there apparently is metabolic stimulation of a tissue (fat) in one species (guinea pig) by an antibody to another tissue (thyroid) of another species (man).

Other instances of metabolic stimulation by antibody

As stated in the introduction, there are other reports of apparent stimulation of metabolic processes by immunoglobulin. For instance, SELL, ROWE and GELL [16] observed stimulation of synthesis of protein, RNA and DNA in rabbit lymphocyte cultures after addition of heterologous antiserum against rabbit serum, and FIDALGO and NAJJAR [17] described enhancement of phagocytosis of bacteria by leucocytes as a property of a fraction of IgG of normal serum. DUMONDE, BITENSKY, CUNNINGHAM and CHAYEN [18] prepared antiserum to ascites tumour cells and found that it unmasked lysosomal acid phosphatase in the cells *in vitro*, in the absence of complement. Perhaps under the same heading of stimulation come reports of

specific antiserum enhancing viability of tumour and skin homografts. Certainly the recent descriptions [e.g. 19] of lymphocyte-transforming activity of antisera to leucocytes exemplify a metabolism-stimulating effect of antibody.

With a view to exploring further the possibility of producing antisera with metabolism-stimulating properties, rabbits were immunized with mouse kidney cells in monolayer culture (kindly provided by Dr. R. Weil). Preincubation of cultured cells with the antiserum (decomplemented by heating to 56° C for 30 minutes) for 14–18 hours led to a progressive enhancement of the incorporation of ^{3}H-thymidine into DNA in the mouse kidney cells. Studies are underway to delineate further the characteristics of the effects engendered by the antiserum, but already this seems to be another example of a phenomenon which is not unique to the thyroid-long-acting thyroid stimulator situation, viz., metabolic stimulation by antibody.

Summary

The long-acting thyroid stimulator is an immunoglobulin that occurs in the blood in Graves' disease. In this presentation it is offered as a prime example of an antibody (to a component of thyroid gland) stimulating the metabolism of its target organ. Stimulation of incorporation of precursor into porcine thyroid RNA and protein *in vitro* by the thyroid-stimulating IgG is described (data of P. R. Adiga). In addition, enhancement of lipolysis of epididymal adipose tissue *in vitro* by addition of thyroid-stimulating IgG (data of I. R. Hart) is described.

Other reported examples of metabolism-stimulation by antibody are reviewed and preliminary results of stimulation of DNA incorporation of ^{3}H-thymidine by mouse kidney cells *in vitro*, on addition of antiserum directed against those cells, is reported.

Zusammenfassung

Das lang wirkende Thyroidstimulans ist ein Immunoglobulin, das im Blut der an Morbus Basedow leidenden Patienten auftritt. In dieser Erscheinungsform dient es als klassisches Beispiel für einen Antikörper (zu einem Bestandteil der Schilddrüse), der den Stoffwechsel des angezielten Organs anregt. Die Stimulation der Aufnahme des Vorläufers in die Schilddrüsen-RNA von Schweinen und in das Protein durch das Thyroidstimulans IgG in vitro wird geschildert (Daten von P. R. Adiga). Außerdem wird die Steigerung der Lipolysis des epididymalen Fettgewebes in vitro durch Zugabe des Schilddrüsenstimulans IgG beschrieben (Daten von I. R. Hart).

Anderweitige Beispiele von Stoffwechselstimulation durch Antikörper werden wiedergegeben, und erste Resultate der Stimulation der DNA-Aufnahme von ^{3}H-Thymidin durch Zellen der Mäuseniere in vitro – unter Hinzugabe von Antiserum in die gleichen Zellen – werden beschrieben.

Résumé

Le stimulateur à action prolongée de la thyroïde est une immunoglobuline que l'on trouve dans le sang des malades atteints de la maladie de Graves (maladie du goitre exophtalmique). C'est un exemple classique d'un anticorps (d'un composé de la glande thyroïde), qui stimule le métabolisme de l'organe dont il est un anticorps. L'auteur décrit comment P. R. Adiga a réussi in vitro à incorporer dans le RNA et les protéines de la thyroïde du porc un précurseur à l'aide de l'IgG qui est un stimulant de la thyroïde. De plus, il décrit comment, selon les travaux de I. R. Hart, l'on peut accélérer la lipolyse du tissu adipeux épididymal in vitro par l'addition de l'IgG stimulateur de la thyroïde.

Puis il passe en revue d'autres exemples cités de stimulation du métabolisme par des anticorps et présente ensuite les résultats préliminaires de la stimulation in vitro de l'incorporation dans le DNA de la ^{3}H-thymidine dans les cellules rénales de la souris.

Riassunto

Lo stimolatore della tiroide ad azione prolungata è un'immunoglobulina che si trova nel sangue dei pazienti affetti da morbo Basedow. In questa forma si presenta come classico esempio di un anticorpo (contro una componente della ghiandola tiroide) che stimola il metabolismo dell'organo bersaglio. Si descrive la stimolazione in vitro dell'incorporazione del precursore nell'acido ribonucleico della tiroide di maiale e nella proteina mediante l'IgG tireostimolante (confronta P. R. Adiga). Si descrive inoltre l'aumento in vitro della lipolisi del tessuto grasso epididimale mediante aggiunta di IgG tireostimolante (vedi I. R. Hart).

Si discutono altri esempi di stimolazione del metabolismo per mezzo di anticorpi e si descrivono i primi risultati sulla stimolazione dell'incorporazione dell'acido desossiribonucleico della ^{3}H timidina da parte di cellule renali del topo in vitro, con l'aggiunta di antisiero diretto contro le cellule medesime.

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