

Hypothesis

ABNORMAL EXPRESSION OF α-GALACTOSYL
EPITOPES IN MAN

A Trigger for Autoimmune Processes?

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Summary 1% of circulating IgG in man is anti-Gal antibody, which interacts specifically with the carbohydrate structure Galα1→3Galβ1→4GlcNAc-R on mammalian glycoconjugates (described throughout as the α-galactosyl epitope). This epitope is abundant on cell surface glycoconjugates of non-primate mammals, prosimians, and New World monkeys. It is not found on cells of Old World monkeys, apes, and man because of diminished α1→3 galactosyltransferase enzyme activity. However, the α1→3 galactosyltransferase gene seems to be present within the human genome. A mechanism that increases α1→3 galactosyltransferase activity in human cells could trigger an autoimmune process mediated by anti-Gal binding to the newly synthesised α-galactosyl epitopes.

INTRODUCTION

ONE of the main characteristics of autoimmune disease in man is the presence of autoantibodies on cells or extracellular components of the damaged tissue. However, with the exception of myasthenia gravis, and perhaps of Graves' disease, the target antigens for the autoantibodies are unknown, and the events which trigger autoimmune processes are poorly understood. I describe a possible mechanism by which abnormal activity of one enzyme, α1→3 galactosyltransferase, may trigger autoimmune processes mediated by the natural anti-Gal antibody.

Natural Anti-Gal Antibody

Anti-Gal is a natural IgG antibody (one apparently produced without active pre-immunisation by the corresponding antigen) which constitutes approximately 1% of circulating IgG in man.¹ Although this natural antibody is polyclonal, it interacts specifically with the carbohydrate structure Ga.α1→3Galβ1→4GlcNAc-R (the α-galactosyl epitope) on mammalian glycoconjugates.²⁻⁴ Anti-Gal can be isolated from AB sera by affinity chromatography with α-galactosyl and melibiosyl adsorbents.¹⁻⁸ Anti-Gal specifically interacts with mammalian glycoconjugates that have chemically defined Galα1→3Galβ1→4GlcNAc residues but does not interact with glycoconjugates with Galα1→4Galβ1→4GlcNAc residues (eg, the P₁ antigen), β-galactosyl residues, or glycoconjugates with other carbohydrate residues.²⁻⁹ In addition, anti-Gal interacts specifically with synthetic Galα1→3Galβ1→4GlcNAc oligosaccharides but not with synthetic Galα1→4Galβ1→4GlcNAc oligosaccharides or with oligosaccharides with β-galactosyl residues.^{2,7,9} (Anti-Gal can bind to the Galα1→6 residue of melibiose and may interact with Galα1→2 residues, but these have not been

SYNTHESIS OF α-GALACTOSYL EPITOPES AND ANTI-GAL
ANTIBODIES IN MAMMALS

Species	Characteristics
Nonprimate mammals Lemurs (prosimians) New World monkeys	Abundant α-galactosyl epitopes on cells. No anti-Gal production
Old World monkeys Apes Man	Diminished expression of α-galactosyl epitopes. Production of anti-Gal (approximately 1% of circulating IgG)

identified in mammalian glycoconjugates.) Anti-Gal is produced throughout life and has been found in similar blood concentrations in neonates and 90-year-old people.¹ The antigenic source for a constant stimulation of anti-Gal production seems to be bacterial strains of the gastrointestinal flora that express α-galactosyl epitopes and readily interact with anti-Gal (eg, *Escherichia coli* and *Klebsiella pneumoniae*).¹⁰

Evolution of the α-galactosyl Epitope

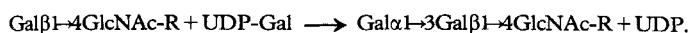
Comparative studies on the distribution of the α-galactosyl epitope and the natural anti-Gal antibody in mammals reveal a striking evolutionary pattern which indicates that suppression of the biosynthesis of these α-galactosyl epitopes might have occurred in ancestral Old World primates after the separation between Old and New World monkeys but before the divergence of Old World monkeys and apes—during the Oligocene period some 25 to 35 million years ago.¹¹ The epitope is abundantly expressed (10⁶–35 × 10⁶ per cell) on cell membranes of non-primate mammals (placental and marsupial), prosimians, and New World monkeys,^{8,12,13} and has been identified both on glycolipids and glycoproteins.^{8,12,13} This epitope has also been found on mouse Ehrlich ascites cells,¹⁴ mouse teratocarcinoma¹⁵ and lymphoma cells,¹⁶ rabbit and cow red blood cells,¹⁷⁻¹⁹ cow thyroid cells,²⁰ and cow thymus cells,²¹ and has been found on N-linked carbohydrate chains of secreted glycoproteins such as mouse laminin^{9,22} and bovine thyroglobulin.²³ In contrast, the α-galactosyl epitope has not been detected on cells or secreted glycoproteins of Old World monkeys, apes, and man^{8,12,23-25} but these species all produce anti-Gal antibodies that comprise approximately 1% of circulating IgG¹² (see table).

These findings indicate conservation of the α-galactosyl epitope during the evolution of many mammals and of ancestral primates but, after the geographic separation of Africa and South America, Old World ancestral primates may have been exposed to an event that suppressed the expression of this epitope. But what could be the cause? One possibility is exposure to a detrimental infectious agent, endemic to the Old World, which expressed carbohydrate epitopes similar to the α-galactosyl epitope. Such an infectious agent might have driven the evolution of Old World primates to the production of anti-Gal antibodies as a protective response, with concomitant suppression of autologous α-galactosyl epitope expression to prevent autoimmunity. Although the causal organism may no longer exist, several infectious agents express α-galactosyl epitopes—retroviruses (eg, Friend murine leukaemia virus²⁶), bacteria (eg, *Klebsiella*, *E coli*,¹⁰ and *Salmonella*²⁷), and protozoa (eg, *Trypanosoma cruzi* and *Leishmania mexicana*^{7,28}). Alternatively, ancestral Old World primates may have been exposed to an infectious agent that exerted its detrimental effect upon α-galactosyl receptors of affected

cells, as does *Clostridium difficile* enterotoxin.^{29,30} Such a hypothetical agent might have induced natural selection for individuals without expression of α -galactosyl epitopes and subsequent loss of immune tolerance to this structure.

$\alpha 1 \rightarrow 3$ Galactosyltransferase Enzyme

The lack of α -galactosyl epitopes in man and Old World primates is caused by diminished activity of the enzyme $\alpha 1 \rightarrow 3$ galactosyltransferase.⁸ In the Golgi apparatus of mammalian cells, N-acetylglucosamine (GlcNAc) residues (Gal $\beta 1 \rightarrow 4$ GlcNAc-R) are usually sialylated by sialyltransferases, which catalyse the synthesis of SA $\alpha 2 \rightarrow 3$ Gal $\beta 1 \rightarrow 4$ GlcNAc-R or SA $\alpha 2 \rightarrow 6$ Gal $\beta 1 \rightarrow 4$ GlcNAc-R residues.³¹ $\alpha 1 \rightarrow 3$ galactosyltransferase can also glycosylate LacNAc residues to form the α -galactosyl epitope, in the reaction:



$\alpha 1 \rightarrow 3$ galactosyltransferase activity has been measured in microsomal fractions of cells from mice,^{15,32,33} rabbits,^{34,35} cows,³⁶ and New World monkeys (marmoset).⁸ However, $\alpha 1 \rightarrow 3$ galactosyltransferase activity has not been detected in cells from man,^{8,35} in which most LacNAc residues are sialylated and lack terminal α -galactosyl residues.

$\alpha 1 \rightarrow 3$ Galactosyltransferase Gene

Is the lack of $\alpha 1 \rightarrow 3$ galactosyltransferase activity in man caused by deletion of the gene from the genome of ancestral Old World primates, or does the gene persist but is not expressed in man because of a specific regulatory-suppressive mechanism?

During ageing of human red blood cells, several hundred cryptic α -galactosyl epitopes are exposed. Binding of anti-Gal to these newly exposed epitopes identifies red blood cells for destruction by reticuloendothelial macrophages.³⁷ These studies indicate marginal activity of the gene in human erythropoietic cells. Kagawa et al³⁸ found that a human lung carcinoma cell line could produce N-glycosylated glycoproteins (recombinant interferon- β) in which a substantial proportion of the carbohydrate chains have the α -galactosyl epitope. Castronovo et al³⁹ have reported the expression of α -galactosyl epitopes on the surface of human mammary carcinoma cell lines and on malignant cells obtained by aspiration biopsy from primary lesions of 50% of patients with mammary carcinoma. Moreover, Lowe et al⁴⁰ have cloned $\alpha 1 \rightarrow 3$ galactosyltransferase cDNA from a mouse cDNA library and have used this cDNA probe in Southern blots to show homologous sequences in DNA from man (personal communication).

These observations indicate that the $\alpha 1 \rightarrow 3$ galactosyltransferase gene persists in man but is suppressed. Under certain conditions, gene suppression may fail and lead to production of the enzyme, which subsequently adds α -galactosyl epitopes to various glycoconjugates.

HYPOTHESIS

Although the mechanism which suppresses $\alpha 1 \rightarrow 3$ galactosyltransferase activity in man remains unclear, I suggest that deregulation of this mechanism, with the subsequent rise in enzyme activity in normal cells, may trigger an autoimmune process. The enzyme would synthesise α -galactosyl epitopes, expression of which on the cell surface would lead to interaction with circulating anti-Gal IgG molecules (fig 1), and bound anti-Gal would start the inflammatory reaction. For example, in the bovine

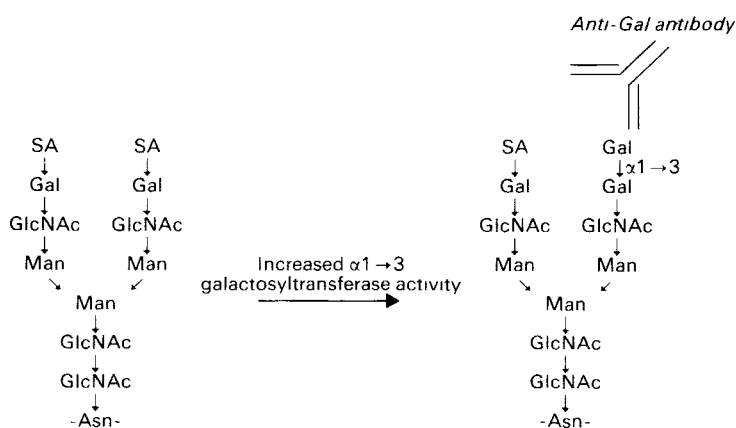


Fig 1—Suggested alteration in glycosylation pattern of N-linked carbohydrate chains on cell surface glycoproteins after increased $\alpha 1 \rightarrow 3$ galactosyltransferase activity.

SA = sialic acid; Gal = galactose; GlcNAc = N-acetylglucosamine; Man = mannose; Asn = asparagine within the protein chain.

thyroid gland the α -galactosyl epitope is abundantly expressed on thyroglobulin molecules²³ and on thyroid cell surface glycoproteins, including TSH receptors.^{20,41} In thyroid cells in man, $\alpha 1 \rightarrow 3$ galactosyltransferase activity is low and LacNAc residues on human thyroglobulin and on thyroid cell surface glycoproteins lack terminal α -galactosyl residues.²³ Increased $\alpha 1 \rightarrow 3$ galactosyltransferase activity within the Golgi apparatus of human thyroid cells would lead to expression of α -galactosyl epitopes on both cell surface glycoproteins and on thyroglobulin (fig 1). Interaction of anti-Gal with these epitopes on thyroid cells would induce a local inflammatory reaction, in which granulocytes and macrophages interact with the Fc portion of the bound antibody. This process may damage physiological barriers and increase exposure of other thyroid gland antigens to the immune system, and lead to expansion of T and B lymphoid cell clones that react with thyroid-specific antigens. Eventually an autoimmune inflammatory reaction, initiated by anti-Gal, would be perpetuated.

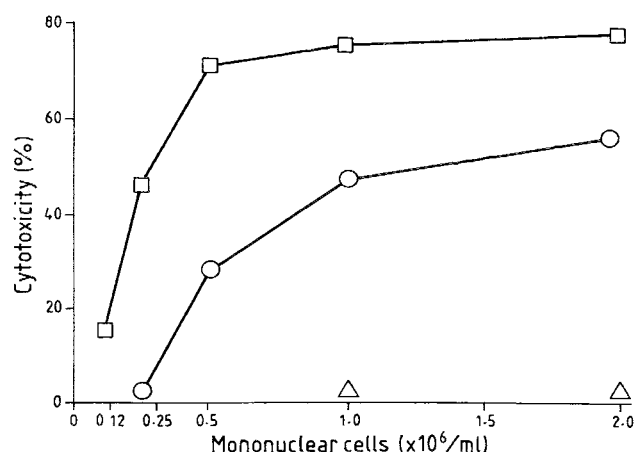


Fig 2—Antibody-dependent cell-mediated cytotoxicity of human mammary carcinoma cell line MCF-7 (O), mouse L cells (□), and human SV-40 transformed fibroblasts (Δ) in presence of 20 μ g/ml of anti-Gal.

Anti-Gal was purified by affinity chromatography on a synthetic Gal $\alpha 1 \rightarrow 3$ Gal $\beta 1 \rightarrow 4$ GlcNAc-R column (Synsorb 115, Chembiomed, Edmonton, Canada). Blood mononuclear cells (isolated on 'Ficoll-Hypaque') were incubated on monolayers of cell lines (0.2×10^6 cells/well) for 40 h at 37°C, after which plates were washed and mononuclear cells and dead cells removed. After trypsinisation, the concentration of live cells was determined and compared to control cell monolayers. No cytotoxicity was observed in the absence of anti-Gal.

Could such a process occur? Casali et al⁴² have found B lymphoid clones that can produce antibodies against human thyroglobulin in healthy people: such clones might participate in an autoimmune process initiated by anti-Gal. Furthermore, LacNAc residues on human thyroglobulin can serve as a suitable acceptor for the synthesis of the α -galactosyl epitope in the presence of the enzyme obtained from cow thymus.⁸ Minimal expression of this epitope—in the order of a few thousand epitopes per cell—may allow sufficient anti-Gal binding to cause antibody-dependent cell-mediated cytotoxicity and thereby generate an autoimmune inflammatory process. The human MCF7 mammary carcinoma cell line³⁹ expresses about 10⁵ α -galactosyl epitopes per cell; sensitivity of the MCF7 cell line to lysis in the presence of anti-Gal is lower than that of mouse L cells which express 6 × 10⁶ epitopes per cell (fig 2).⁸ Nevertheless, in-vitro studies indicate that anti-Gal antibody can induce lysis of MCF7 cells at one fifth of its normal concentration in the circulation. Therefore minimal activation of the α 1→3 galactosyltransferase gene may be sufficient to produce a threshold number of α -galactosyl epitopes for an autoimmune response to be started in susceptible cells. Such activation could be caused by factors—such as viral infection, irradiation, or chemicals—which might impair the suppressive mechanism that prevents α 1→3 galactosyltransferase expression. After irradiation of the human cell lines HL-60 and HeLa, for example, subclones of cells which at least temporarily express α -galactosyl epitopes can be isolated.

The mechanism which I describe could occur in any tissue in which cells might express the α -galactosyl epitope as a result of abnormal α 1→3 galactosyltransferase activity (eg, synovial membranes, Langerhans islets, and erythropoietic cells). A similar autoimmune process may involve extracellular proteins such as laminin: in mouse laminin, there are 50–70 α -galactosyl epitopes per molecule,⁴³ but LacNAc residues in human laminin are not α -galactosylated.²⁴ Increased α 1→3 galactosyltransferase activity in the glomerular cells which produce laminin within the kidney may lead to autoimmune glomerulonephritis mediated by anti-Gal binding to the abnormally galactosylated laminin. Studies by Etienne-Decerf et al⁶ and Davin et al⁵ have indicated possible anti-Gal involvement in autoimmune thyroiditis and glomerulonephritis, respectively.

How might evidence be obtained to support this hypothesis? Isolation of α -galactosyl glycoconjugates from diseased autoimmune tissues (eg, by affinity chromatography with anti-Gal) would help, but direct identification may be difficult because of the paucity of α -galactosyl epitopes in tissues affected by autoimmune diseases, and the delay between the start of the autoimmune process and diagnosis. Alternatively, α 1→3 galactosyltransferase gene expression in man could be studied, and with cloned α 1→3 galactosyltransferase cDNA, it should be possible to determine whether suppression of α 1→3 galactosyltransferase occurs at or after transcription, and to study the mechanisms that cause deregulation of α 1→3 galactosyltransferase activity. If deregulation occurred at transcription, use of appropriate oligonucleotides may enable tissues to be screened by the polymerase chain reaction to assess the contribution of α 1→3 galactosyltransferase to autoimmune diseases in man.

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Reviews of Books

Starling's Law of the Heart Revisited

Developments in Cardiovascular Medicine, vol 89.—Edited by H. E. D. J. ter Keurs and M. I. M. Noble. Dordrecht: Kluwer. 1988. Pp 141. £43.25. ISBN 0-89838382X.

MOST doctors retain at least a memory of “Starling’s Law” from their undergraduate physiology days. Although it is 75 years since Starling obtained his experimental data which, in combination with earlier observations of Frank, provided the basis for formulation of his law, it remains the foundation of our understanding of cardiac dynamics.

Since Starling’s time, many people have tried to elucidate the mechanism whereby pressure development and cardiac output increase as ventricular end-diastolic volume and fibre length increase. Dr ter Keurs and Dr Noble here bring together expert accounts of current understanding of the Frank-Starling mechanism, and the book draws its authorship from speakers at a series of recent symposia held in the UK. Repeatedly we are reminded of the power of the techniques now available, including simultaneous measurement of tension and intracellular free calcium concentration by means of calcium-sensitive photoproteins, the use of laser diffraction techniques to enable instantaneous measurement of sarcomere length, and especially the determination of length-tension curves in single cardiac myofibrils (by Iwazumi)—a technical *tour de force*.

Several contributors provide evidence for the current belief that the Starling mechanism is in large part a result of increased affinity for calcium of the protein troponin, as muscle fibres lengthen, rather than increased intracellular calcium release. A chapter by Arts and Reneman offers a model of ventricular contraction in which the mathematical derivations may elude many of us, but it is reassuring to know the model “is equally applicable to mice and elephants”.

For whom is this book intended? The chapters have been written by experts, largely without compromise, with similar specialists in mind. It will thus be of use primarily to cardiac physiologists and those in allied disciplines. Nonetheless, several chapters should appeal to a wider readership. Elzinger and colleagues present a lucid and refreshing account of cardiac pump function and adaptive hypertrophy approached from engineering criteria; this

should be required reading for all those now attempting to explain cardiac hypertrophy as a consequence of activity of sympathetic nerves, or of cellular growth factors. The final chapter, by Noble, will fascinate many cardiologists, not least because of its clear discussion of appropriate and inappropriate indices of myocardial contractility, and also its impassioned plea to abandon the terms “preload” and “afterload”.

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The Autopsy—Medical Practice and Public Policy

Rolla B. Hill and Robert E. Anderson. Boston: Butterworths. 1988. Pp 294. £45. ISBN 0-409901377.

THE numbers of (non-forensic) hospital necropsies continue to fall worldwide. There is no single reason for this decline and no single remedy for it. The postmortem examination is increasingly seen as an expensive and vestigial activity, rooted in the past and contributing little to the daily practice of modern clinical medicine. Some counter-arguments are now provided by Dr Hill and Dr Anderson; although strong advocates of necropsy, they analyse rigorously the reasons for its decline, a process to which clinicians and pathologists are shown to have made nearly equal contributions. The evidence reviewed shows that necropsies disclose about 10% of diagnostic discrepancies that are sufficiently large to have had an adverse effect on the patient’s survival. This figure is remarkably consistent and, apparently has not fallen despite the introduction of new diagnostic techniques. A “partial list of diseases diagnosed or critically clarified through autopsy since 1950” makes exaggerated claims, but elsewhere the authors’ emphasis on postmortem examination as a valuable source of information on environmental and iatrogenic disease, and their plea for more accurate mortality statistics, are difficult to refute.

However, necropsy may make its most valuable contribution as a method of quality assessment, and it is perhaps in this context that the future role of the non-forensic examinations can be most clearly discerned. Such considerations may conceivably be recognised by politicians and health administrators who, according to Hill and Anderson, “bemoan the need for autopsies, encourage under-utilisation and look for ways to cut the autopsy

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