*Glycoconjugate Journal* (1997) **14:** 159–173

REVIEW

# Blood group antigens: molecules seeking a function?*

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**The blood group antigens have been dismissed by some researchers as merely 'icing on the cake' of glycoprotein structures. The fact that there are no lethal mutations and individuals have been described lacking ABO, H and Lewis antigens seems to lend weight to the argument. This paper reviews the research which suggests that these antigens do indeed have function and argues that blood group antigens play important roles in modulation of protein activity, infection and cancer. It explores the evidence and poses questions as to the relevance and implications of the results.**

***Keywords*: blood group antigens, glycoproteins, carbohydrates**

## The past

### Discovery of the A, B and H antigens

The ABO blood group system was first described by Karl Landsteiner [1] in 1900. He mixed the blood of members of his department and made the fundamental observation that some bloods when mixed together formed clumps whereas other bloods did not. This led to the suggestion that there were three blood group antigens, A, B and O. Individuals who lacked an antigen had, in their sera, natural antibodies that had the ability to agglutinate cells carrying that antigen. The blood type AB was subsequently discovered by Decastello and Sturli [2] (Table 1).

The name 'blood group antigens' stems from this work; the antigens were present on red cells and this discovery led to the development of safe transfusions of blood from one person to another. The work of Grethe Hartmann [3] in the 1940s was probably the first to demonstrate that these antigens were not confined to the red cells but were present on other cell types and in secretions. Currently, there is a move to be more accurate and use the more correct nomenclature 'histo-blood group antigen'. The system seemed uncomplicated; three antigens, six genotypes and four phenotypes (Table 2). However, within 30 years of their original discovery the sub-groups of A; $A_1$, $A_2$ and $A_3$ had been recognized [4, 5]. Since that time, the ABO system has been shown to be highly polymorphic: this has led to the idea that these antigens are 'unimportant' since there appears to be no 'lethal' polymorphism.

At first the *A*, *B* and *O* genes were all thought to be functional, each giving rise to an antigenic product. Antibodies existed which would agglutinate A or B cells but would not agglutinate group O. The product of the *O* gene was thought to be the O antigen that was detectable with a range of lectins. However, pioneering work in the department of Professor Walter Morgan and Winifred Watkins began to suggest that the 'O' antigen was in fact present in not only O individuals but also to a lesser extent in B, A and AB individuals. This led to the suggestion that the 'O' antigen was in fact a precursor of the A and B antigens and that the *O* gene was a silent allele at the *ABO* locus. The 'O' antigen was then termed the 'H' antigen [6].

### The structure of the A, B, H and Lewis antigens

The nature of the biosynthesis and the inter-relationships between the ABO and H systems became clear when the structure of the antigens was determined (Figure 1). The antigenic potential was not affected by treatment with protease but was affected by periodate. This indicated that the antigens were carbohydrate in nature rather than protein. Structural analysis was carried out on blood group substances purified from ovarian cyst mucin; this provided an abundance of an easily purified form of the antigens. The analyses, carried out using the tools available; exoglycosidases, inhibition of serological reactions and chemical analyses, led to the determination of the structures of the antigens and the nature of the immunodominant sugars [7–24].

It was clear that the H determinant formed part of the A and B determinants and that A and B differed only with respect to the terminal sugar: in the case of the A

* Presented at the first Electronic Glycoscience Conference (EGCI) on the World Wide Web, September 1995.

0282–0080 

**Table 1.** Relationship between red cell antigens and serum antibodies.

| *Antigen* | *Antibody* |
|---|---|
| O | anti-A and anti-B |
| A | anti-B |
| B | anti-A |
| AB | neither |

**Table 2.** Blood group genotypes and phenotypes.

| *Genotype* | *Phenotype* |
|---|---|
| *OO* | O |
| *AO* | A |
| *AA* | A |
| *BO* | B |
| *BB* | B |
| *AB* | AB |

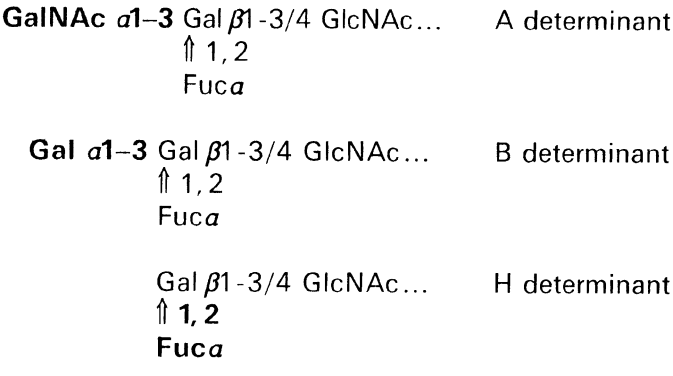


**Figure 1.** The structure of the A, B and H determinants. The immunodominant sugar is highlighted.

determinant the immunodominant sugar is *N*-acetyl-D-galactosamine and in the case of the B determinant it is D-galactose. These two sugars differ only in the substitution at C2 where the N-acetyl-D-galactosamine has an N-acetyl-amino group whereas D-galactose has a hydroxyl group. Antibodies that differentiate between the A and B determinants were therefore presumed to recognize this difference in the terminal sugars.

The fucosyl residue was important for reactivity of these antigens with their respective antibodies but the reactivity with H-specific lectins was lost when the terminal sugars were added. Work on red cell antigens had been relatively straightforward but it was apparent that the situation in secretions differed. Some individuals of known ABO blood type were found to secrete 'blood group substances' in secretions such as saliva, tears and gastric mucosa [25, 26]. However, a

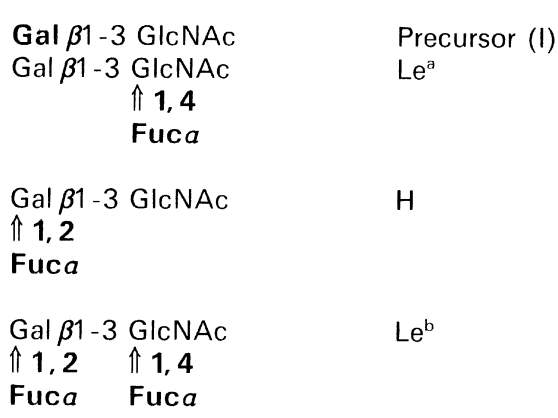


**Figure 2.** Structure of H and Le antigens.

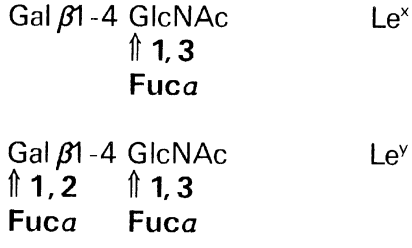


**Figure 3.** Structure of the Lewis related structures. The immunodominant sugars are highlighted.

percentage of the population, 20% of Caucasians, secreted substances devoid of these antigens. This led to the idea that there was a locus controlling the expression of the H antigen in secretions and the genes involved were named *Se* (secretor) and *se* (non-secretor). Although originally thought of as a 'modifier' of expression of the *H* gene, it has become accepted that *Se* is a structural gene, showing strong homology to the *H* gene [27]. The *H* and *Se* genes differ with respect to their tissue specific $Le^a$ expression [28–30].

The relationship between ABO, H, Se and the Lewis [31, 32] blood group systems was only solved when the Lewis antigens were characterized [33, 34]. It had been observed that individuals whose red cells typed as Le(a − b +) were always secretors, individuals who typed as Le(a + b −) were always non-secretors and that individuals who typed as Le(a − b −) could be either secretors or non-secretors [35, 36]. Additionally, it was shown that the Lewis antigen present on the red cells was in fact not an integral part of the red cell membrane but was absorbed from the serum [37, 38].

The $Le^a$ antigen is formed from the precursor antigen and does not require expression of the H antigen. The $Le^b$ antigen on the other hand requires both the H and $Le^a$ antigens to be expressed to give rise to the hybrid $Le^b$ structure (Figure 2). Thus, if the Lewis antigens are in fact

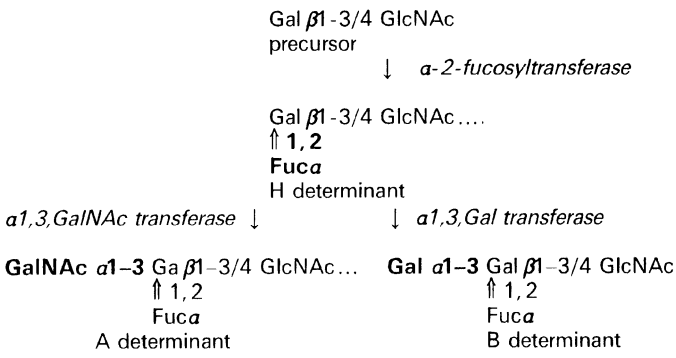


**Figure 4.** Biosynthesis of A, B and H antigens.

made in secretory tissue then their expression is reliant on the expression of the H antigen which in turn is affected by the presence or absence of the *Se* gene. Individuals lacking the *Le* gene, *ie* Le(a − b −) may be either secretors or non-secretors. An analogous system involving type 2 chain precursors is responsible for the production of $Le^x$ and $Le^y$ determinants [Figure 3].

## Biosynthesis of blood group antigens

Once the structures of the antigens were defined, and it was obvious that the removal of single sugars destroyed antigenic specificity, Winifred Watkins suggested that the synthesis of these oligosaccharides occurred through the action of glycosyltransferases. In 1959 she published a biosynthetic pathway for the production of the A, B and H antigens [39] that was subsequently proven when the relevant enzymes were characterized [40–46].

### *Enzyme specificity*

The gene products are glycosyltransferases which add sugars sequentially to a precursor chain (Figure 4). The enzymes utilize nucleotide sugar donors and were thought to be absolutely specific with respect to donor and acceptor substrates. Early work showed that the enzymes would only add sugars on to specific acceptors, for example, the α-2-fucosyltransferase cannot utilize substrates analogous to $Le^a$ and the *A* and *B* transferases can only use substrates which have the H determinant present. However, they cannot use $Le^b$ related structures as acceptors.

Nucleotide sugar specificity was thought to be as strict. In the late 1970s, in an experiment designed to address the problems of enzyme co-operativity a small amount of A tetrasaccharide was produced, after lengthy incubation, by a serum from a known 'strong' blood group B donor. Nearly 100 more group B sera were then tested under similar conditions and it was found that all had the potential to make A determinants, albeit at a low rate [47, 48]. The product was subsequently characterized and shown to be authentic A tetrasaccharide [49]. It was also demonstrated that the *A* transferase could utilize UDP-galactose as a sugar donor to make B determinants [50, 51] with a low efficiency and that there appeared to be enzymic activity associated with group O serum which could also synthesize group A determinants [52]. So, specificity for acceptor appears to be more critical than for nucleotide sugar. The *A* and *B* genes are alleles at a single locus on chromosome 9 [53] and therefore one would expect that the enzymic products would be structurally very similar. Both have identical acceptor substrate requirements and differ only with respect to their nucleotide sugar donor. The difference between the two sugars transferred is significant, but the binding sites, though different, can bind either donor although with a vastly different affinity. Recently the lack of absolute nucleotide sugar specificity of another glycosyltransferase has been reported; Do and co-workers have shown that α-lactalbumin-induced bovine milk β-1,4-galactosyltransferase can utilize UDP-N-acetylgalactosamine [54].

The enzymes synthesizing the Lewis and the Lewis-related determinants have been characterized and biosynthesis follows a similar pattern [42, 55] (Figure 5). In secretory tissue and on red cells, since the Lewis antigens are absorbed from serum, the expression of Lewis and Lewis-related antigens is reliant on the presence of the H antigen whose expression is determined by the *Se* gene. In tissues in which the *H* gene (Table 3) determines expression of the H antigen, $Le^a$ and $Le^b$ antigens may be expressed in non-secretor individuals even though the antigens are not detectable on either red cells or in secretions.

# The present

## The enzymes and their genes

The genes encoding the glycosyltransferases were difficult to clone since the levels of protein expressed were so low, sub-microgram in tens of litres of serum, and human tissue was difficult to obtain in sufficient quantities for enzyme purification. The first approaches involved attempts to produce polyclonal and monoclonal antibodies to the enzymes

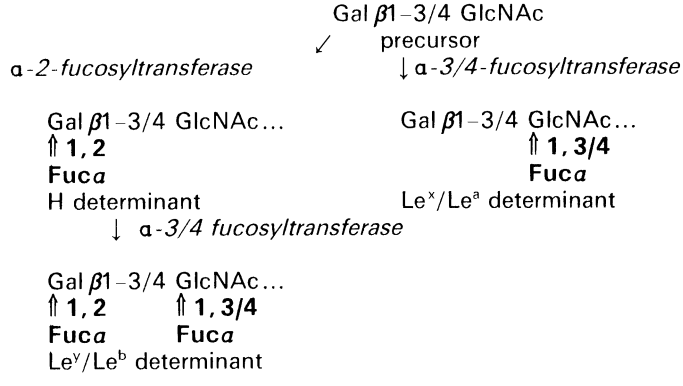


**Figure 5.** Biosynthesis of fucosylated antigens.

**Table 3.** Effect of *H*, *Le* and *Se* genes on antigen expression.

| H *status* | Se *status* | Le *status* | *Antigens in secretions* | *Antigens on red cells* | *Antigens in non secretory tissue* |
|---|---|---|---|---|---|
| *Hh*/*HH* | *SeSe*/*Sese* | *LeLe*/*Lele* | H, Leb | H, $Le^b$ | H, $Le^b$ |
| *hh* | *SeSe*/*Sese* | *LeLe*/*Lele* | H, Leb | $Le^a$ | $Le^b$ |
| *Hh*/*HH* | *sese* | *LeLe*/*Lele* | Lea | H, $Le^b$ | H, $Le^a$, $Le^b$ |
| *hh* | *sese* | *LeLe*/*Lele* | $Le^a$ | $Le^a$ | $Le^a$ |
| *Hh*/*HH* | *SeSe*/*Sese* | *lele* | H | H | H |
| *hh* | *SeSe*/*Sese* | *lele* | H | weak H | – |
| *Hh*/*HH* | *sese* | *lele* | – | H | H |
| *hh* | *sese* | *lele* | – | – | – |

**Table 4.** Landmarks in cloning *ABO*, *H*, *Se* and *FUT* genes.

| | |
|---|---|
| Sepharose 4B purification of *A* transferase | [81] |
| Purification of *B* transferase | [82] |
| Preparation of polyclonal anti-*A* transferase | [81, 83, 84] |
| Preparation of monoclonal anti-*A* transferase | [85] |
| Expression of *A* transferase in *Xenopus* oocytes | [86] |
| Cloning *ABO* genes | [57, 58, 87–89] |
| Cloning *H*, *Se*, *Le* and *FUT* genes and expression of *FUT* genes *in vitro* | [62–75] |

(Table 4); these were then used to screen expression libraries [56]. The use of polyclonal antibodies in library screening is fraught with problems, the most important of which is the presence of contaminating antibodies; the antibody is only as specific as the immunizing protein is pure. Many of the monoclonals raised were IgM and were found to recognize carbohydrates rather than proteins.

Yamamoto and his colleagues finally cloned the genes at the *ABO* locus by purifying enough enzyme to sequence and using synthetic degenerate probes to screen libraries. This resulted in the publication of the cDNA sequence and partial genomic sequence [57–59]. The *A* and *B* genes were shown to differ with respect to four bases: two of which cause changes in amino acids and are therefore responsible for differences in specificity. Three *O* alleles have now been described in which mutations have occurred that result in the effective absence of gene product [57, 60, 61]. The *Le*, *H* and *Se* genes were then cloned by Lowe and his colleagues in what can only be described as a 'tour de force' [62–69]. The cloning of the fucosyltransferase genes [70–74] has certainly helped to clarify the 'muddy waters' in an area which has for so long been a subject of intense debate. The cloning of the *H* and *Se* genes has proven Oriol's theory that *H* and *Se* were both structural genes resulting in the production of two α-2-fucosyltransferases which differ in acceptor specificity and tissue specific expression [27, 75].

What has the cloning told us about the enzymes? Currently the answer must be 'a lot about the gene structure and the relatedness of genes' but 'not much about the protein structure and function'. Maybe we expected too much from the cloning of the genes. The work has been difficult and this is reflected in the current paucity of information about the proteins themselves. The next decade may well see a huge expansion of our knowledge in this area. The researchers involved in the cloning have provided us with a resource whose importance cannot be underestimated. Of the genes themselves and their relationships, much interesting information has been gleaned. For example, the presence of the locus encoding production of 'straight chain' B (Galα1, 3 Galβ-⋯) close to the *ABO* locus on chromosome 9 [76] suggests that these two genes may be the product of gene duplication and subsequent mutation. This situation is analogous to that of the *H* and *Se* genes. Man and the higher apes do not express the 'straight chain' gene due to a specific mutation whereas this is the predominant antigen in most other species [77–80]. The structure synthesized by this gene has provided a headache for those interested in the production of therapeutic monoclonal antibodies and recombinant proteins since all humans possess natural antibodies to this structure. In addition, those who have tried to clone the blood group genes in non-humans have been bedevilled by problems of cross-hybridization. In the field of transgenic animals for xenografting, work is already underway to alter the expression of this gene; thus avoiding xenotransplant rejection.

## What are the functions of the ABO, H and Le genes?

What is the function of the blood group antigens? Indeed do they have to have a function? Nature does not always eliminate useless genes and since there is no disadvantage to their presence or absence perhaps they are not worth removing. However, the *ABO* genes are present in monkeys and this suggests that the polymorphisms evolved more than 13 million years ago. The enzymes and/or antigens of the *ABO*, *H*, and *Le* systems have been reported in sources

as diverse as pigs [90], toads [86], plants [91, 92] and bacteria [92, 93]. Surely they cannot have been maintained for so long if they have no purpose?

We need to determine whether there is evidence that these genes have function and ask how we could 'firm up' these data or indeed devise methods of testing the hypotheses. We must be careful not to be seen to be inventing function simply to make carbohydrates more interesting or more worthy of funding. Such strategy may give us instant gain but ultimately will be our downfall. There are four main areas to be looked at; the association of blood groups with predisposition to infectious disease; the role of the antigens in cancer; the effect of blood type on susceptibility to multifactorial conditions such as heart disease and auto-immune disease; the effect of differential glycosylation on specific glycoproteins.

## Blood group antigens and infectious diseases

The blood group antigens are expressed maximally in mucins that are found on the 'exposed' surfaces of the body, such as the mouth, gastro-intestinal tract, the urogenital tract and the lungs. The mucin is thought to have a protective role and one possible function of the blood group antigens is the binding of micro-organisms within the mucin for their subsequent removal by cells of the immune system. So, is there evidence for such a role? It has been shown that the *ABO*, *H/Se* and *Le* genes are associated with a range of infectious diseases including typhoid and plague. Indeed, this relationship between blood groups and diseases is not a new idea, Springer and his colleagues in the 1950s and 1960s published data suggesting that the naturally occurring anti-A and B antibodies were raised in response to bacterial infection. Specifically, he fed germ-free chickens *Escherichia coli* O86 or placebos and monitored the serum levels of anti-B. He proved that ingestion of *E. coli* caused the production of anti-B. His explanation was that the *E. coli* O86 carried B antigen on its cell surface and this acted as an immunogen. The antibody formed reacted with group B cells and B antigen analogues but not with other related oligosaccharides [94]. Similar experiments were then carried out with human subjects and yielded essentially the same results [95]. This work was dismissed as an interesting curiosity; surely we all know that bacteria do not produce glycoproteins and could not have antigens similar to humans? I hope Springer said 'I told you so' when he read the report in 1985 which showed that within the polysaccharide of the *E. coli O86*, there was what is undoubtedly B antigen [96]. Subsequently strains of *E. coli* have been shown to possess H antigen on their cell surfaces [97], presumably giving a possible explanation for the production of anti-H in the rare Bombay and paraBombay variants who do not express the H antigen.

So, is there any credence in the often dismissed work suggesting that the distribution of ABO blood groups world-wide can be associated with the epidemics of, for example, plague and influenza. The idea that influenza carried A antigen was put forward [98] with the hypothesis that non-A individuals produce anti-A antibodies and therefore can clear infection readily whereas group A individuals have no protection. Mourant [99] showed apparent correlation between the spread of ABO blood groups and these infections in communities world-wide. However, reports refuted the claims that influenza carried A antigen and stated that the A antigen in the viral preparation used in these experiments was contaminated with A substance absorbed from the growth media. Here the story ended, only to be re-examined this decade following renewed reports of associations between bacteria and viruses and blood groups. The associations may be more complex but ABO and H blood groups do appear to be intimately associated with susceptibility to infectious agents (Table 5).

The most disconcerting aspect of this work is that it is heavily reliant on statistics and for some organisms, for example *Helicobacter pylori*, associations have been published by some workers and apparently refuted by others [100–104]. What is the hard evidence? There are obvious associations between the current world-wide distribution of blood groups and earlier epidemics of infection that suggest either a susceptibility to infection or a protective factor associated with blood type. The preferential binding of *Pseudomonas aeruginosa* to blood group A ties in well with the already proven link between blood group A and the susceptibility to glue ear [105]. However, many of the associations may be multifactorial in nature; the blood type may be one of a number of predisposing factors all contributing to the eventual disease. The answer must surely lie in a systematic evaluation of the interactions of microbes with blood group antigens and an investigation into the link between blood group antigens, mucins, bacterial polysaccharide antigens, microbial lectins and adhesins.

The blood group antigens are known to provide a food source for bacteria and protozoan parasites of both the gut and the urogenital tract. Hoskins and colleagues [122] have shown that colonic microbes may adhere to and metabolize blood group antigens and work presented by Connaris shows that a number of protozoans that inhabit mucin-rich environments produce glycosidases [123]. It is not completely clear whether their function is purely to provide food for the organisms, or whether the glycosidases may also play a role in pathogenicity, destroying the integrity of the mucin facilitating its enzymolysis.

## Blood group antigens and cancer

The relationship between blood groups and cancer may at first appear much less complicated than their relationship with infectious disease. However, there is as much confusion in this area. The problem lies with the natural tissue-specific

**Table 5.** Infections associated with specific blood group antigens.

| *Organism* | *Disease* | *Blood group* | *Evidence* |
|---|---|---|---|
| *Helicobacter pylori* | Gastric ulcers | O | Statistics and lectin binding [100–105] |
| *Neisseria sp.* | Meningitis | Non-secretors | Statistics [106] |
| *Plasmodium falciparum* | Malaria | A, B, AB | Rosetting, lectin mediated binding [107] |
| *Leishmania donovani* | Leishmaniasis | ABO | Family studies [108] |
| *Salmonella typhi* | Typhoid | B | Family studies [109] |
| *Filaria sp.* | Filariasis | B | Statistics [110] |
| *Candida albicans* | Peptic ulcers and oral candidiasis | Non-secretors | Statistics and adhesion studies [111–113] |
| *Smallpox* | Smallpox | A | Statistics [114] |
| *Pasteurella pestis* | Plague | O | Statistics [115] |
| *E. coli* | Enterotoxoid mediated cholera | A and B | Binding studies [116, 117] |
| *Pseudomonas aeruginosa* | Glue ear | A | Statistics and adhesion studies [105–118] |
| *E.coli* | Urinary tract infection | Le(a-b-) and non-secretor | Statistics [119–121] |

expression of the antigens. For example, the blood group ABH antigens are expressed to different degrees in the gut. In the stomach expression is maximal whilst in the colon it is minimal. This is due to the tissue specific expression of the *Se* and *H* genes [124].

It is a 'rule of thumb' that if antigens are expressed in the adult they are absent in the foetus and vice versa. The antigens have been termed onco-developmental in as much as the expression in cancerous tissues mimics that of the foetus. In gastric cancers [125], there is loss of blood group A, B, H and $Le^b$ antigens and a concomitant increase in $Le^a$, however, in colonic cancer A, B, H and $Le^b$ antigen expression is vastly increased [126–129]. In both cases it is the α-2-fucosyltransferase produced by the *Se* gene which is affected [125, 130, 131]. In bladder cancer, however, the modulation of the activity of the α-*N*-acetyl-D-galactosaminyltransferase is crucial [132, 133], whilst in leukaemias [134–137] and sarcomas (Greenwell, unpublished results) the *H* gene specified α-2-fucosyltransferase and the *A* and *B* transferases may be affected. The final repertoire of antigens expressed by a particular carcinoma is the result of the exposure or presentation of terminal sugars not normally detected in that tissue (Table 6).

**Table 6.** Tissue specific changes in blood group expression in carcinomas.

| *Tissue* | *Change* | *Reference* |
|---|---|---|
| Colon | Re-expression of ABH | [126, 130, 138–141] |
| Gastric | Loss of ABH, re-expression of $Le^a$ | [125, 142, 143] |
| Bladder | Loss of ABH, appearance of $Le^a$ | [144, 145] |
| Lung | Loss of ABH | [146] |
| Breast | Loss of H and $Le^b$ | [147, 148] |
| Cervix | Topographical not quantitative | [149] |
| Liver | Increased ABH | [150] |
| White cells | Decreased ABH | [134–136] |
| Squamous | Decreased H | [151] |
| Endometrium | Appearance of ABH | [152, 153] |
| Epithelium | Expression of ABH | [154] |
| Head and neck | Loss of ABH | [155] |
| Pancreas | Topographical changes in Lewis expression | [156] |

There are no generalized patterns of changes in antigen expression associated with cancer. It has been noted, however, that there is often aberrant expression of blood group A antigen in cancers of non-A patients. These antigens, which are present in localized foci of particularly gut carcinomas, have been fully characterized and are 'normal' A antigen [157–160]. How can we explain the apparent *de novo* synthesis of these antigens in the absence of the genes associated with their synthesis? Recently Clausen and his colleagues have suggested that point mutations may occur in the *O* gene, restoring the reading frame leading to production of an *A*-like transferase [161, 162]. Other alternative explanations are, use of a second initiation site in the *O* gene which results in the production of a truncated yet enzymically active protein or the activation of a pseudo- or related gene. In 1987 we described [52] the detection of *A* transferase activity in a purified preparation of group O serum.

Subsequent work has shown a low but detectable amount of *A*-transferase-like activity in about 60% of O sera tested (Table 7). Whether this is indeed the product of the *O* gene is

**Table 7.** *A* transferase activity in group O sera.

| *Number of samples* | *Enzyme activity pmol h $^{-1}$ ml $^{-1}$ serum* |
|---|---|
| 9 | Undetectable |
| 9 | 0.1–1 |
| 4 | 1–4 |
| A control | 1625 |

still unclear. However, the following evidence has been gathered; 60% of group Os tested have very low expression of an *A*-like transferase; the enzyme binds to Sepharose 4B, a specific ligand for the *A* transferase; pH and cation requirements are similar to the $A^2$ transferase; the purified enzyme reacts with a specific polyclonal anti-*A* transferase in enzyme precipitation experiments; substrate specificity is identical to that of the $A^2$ transferase; the product of the reaction has been identified by chromatographic mobility and glycosidase degradation.

In the light of the increased incidence of many cancers in group A [Table 8] and the aberrant expression of the A antigen in group O and B individuals with cancer [157–162], is there evidence that the A antigen plays a role in cancer? Since the difference in structure between the A and H antigen is a single *N*-acetyl-D-galactosamine group, we need to explore the effect of the change of a single sugar on the glycoprotein molecules associated with the carcinogenic process. Work published by De Fize and coworkers [163] suggested that addition of a single *N*-acetyl-D-galactosamine group to the Epidermal Growth Factor Receptor (EGFR) significantly affected receptor turnover, high affinity binding of growth factor, protein phosphorylation and tyrosine kinase activity. This finding is corroborated by the study carried out by Engelmann and colleagues [164] who analysed the nature of EGFR on red cells from group A and O donors. This work clearly showed that there were no differences in the dissociation constants but that group $A^1$ red cells had an increase in the number of high affinity binding sites compared to red cells of group O or B. Growth factors are intimately involved in cancer processes and this change in the receptor may point to a mechanism whereby group A individuals are more susceptible to cancer. The suggestion therefore is that the addition of a single sugar can radically change the conformation or charge on a glycoprotein molecule so as to alter the binding kinetics of a growth factor to its receptor. The fact that EGFR contains a truncated form of the amino acid sequence encoded by the oncogene v-erbB is interesting since Idikio and Manickavel [165] have suggested that loss of blood group antigens in prostatic cancer may be correlated to increased expression of v-erbB and increased malignancy. This prompts the question 'Does erbB carry ABH determinants which modulate its activity?'

**Table 8.** Cancers associated with blood group A.

| *Cancer* | *Blood group affected* | *Reference* |
|---|---|---|
| Rectal | A | [166] |
| Cervical | A | [167] |
| Pancreatic | A | [168] |
| Leukaemia (ALL) | A2 | [135] |
| Gastric | A | [169] |
| Breast | A | [170] |
| Ovarian | A | [171] |

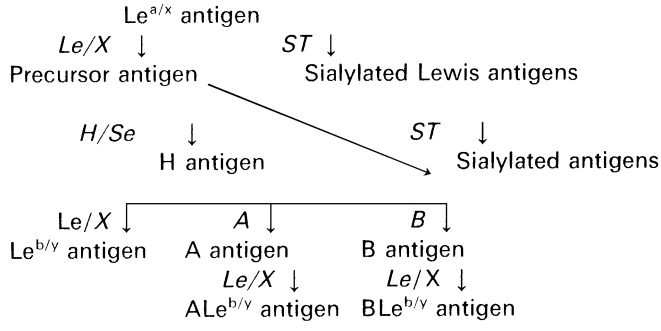


**Figure 6.** Possible scenarios for abnormal antigen expression.

Work carried out by Jacques LePendu and his colleagues provides intriguing evidence that glycosylation may affect the immune recognition of cancer cells by natural killer (NK) and LAK cells. In experiments in rats his group were able to show that cells carrying the A antigen were much less susceptible to the action of NK and LAK cells than were those carrying the H antigen. Additionally, cells carrying the precursor chains alone were most susceptible [172, 173]. This may tie in well with both the increased chance of developing tumours in group As and the finding that some tumours are more common in non-secretors.

The changes in the expression of the blood group A, B, H, Se and Le antigens may affect or be affected by changes in other glycosyltransferases which compete for the same precursor substrates [174, 175]. The specificity of the enzymes for their substrates means that if a competing enzyme adds a sugar to the precursor 'out of order' then the normal repertoire of immunodominant sugars may not be added. An understanding of the mechanisms involved in the production of the overall cancer-antigen profile requires analyses of the antigens, the enzymes and the genes. We need to look at the full range of enzymes and their cellular localization in order to clearly appreciate the story (Figure 6).

We should also address the problem of the effect of changes in glycosylation on the activities of key enzymes and proteins involved in regulation of cell growth and differentiation. If, for example, the binding of EGF to EGFR is different in blood group A and O, loss or gain of expression of the A antigen may significantly alter the

binding properties of that protein affecting the cell signalling and growth. Are changes in blood group antigen expression a cause or effect of cancer? A number of 'pre-cancerous' conditions have also been shown to have altered blood group antigen expression [136, 138, 142, 143, 175–179]. Fujimoto and Miyano were able to demonstrate $Le^b$ antigen expression in colonic crypts of patients with Hirschsprung disease [180]: this disease leads to enterocolitis and colonic cancer. This work suggests that re-expression of the α-2-fucosyltransferase is an early event in carcinogenesis. A number of similar examples are also suggestive of an early change in blood group antigen expression in cancer. In my laboratory (Greenwell, unpublished data) we have carried out a long term study of patients with sarcomas in whom A and B antigens were lost from the red cells. Chemotherapy and radiotherapy were used to effect a cure, at which time the A and B antigens were once more detected on the red cells. This suggests that blood group antigen expression may be used as an indicator of successful therapy. Similar results were obtained with some leukaemic patients. Changes in expression of carbohydrate antigens can be used as markers for disease progression and prognosis (Table 9). In all the cases shown, loss of A, B or H antigens appears to be associated with enhanced malignancy and in many cases is one of the most reliable markers of disease status.

Will the advent of molecular biology help us to understand the changes we see in antigen expression? David's group has looked at A antigen production in carcinomas of individuals who had been genotyped as normal *O* [181]. A monoclonal antibody raised against the *A* transferase recognised enzyme in these patients. This provided definitive proof of A antigen production, but not an explanation of the mechanism. There have been suggestions that new mutations may impart A enzyme activity in group Os. However, it is not clear how such a mutation could be a common feature of intestinal carcinomas.

## Blood group antigens and multifactorial diseases

Blood type has been associated with increased risk of heart disease. Statistically group A individuals are more likely to suffer from myocardial infarction and cardiovascular disease than group Os [182–184]. Additionally, there is a link between blood type A and systolic blood pressure in Caucasians, but not Blacks, and blood group As have also been shown to have higher cholesterol levels and β-lipoprotein cholesterol (LDL). Interestingly, Fox and his colleagues [185] have stated 'White adolescents of A phenotype have a significantly greater risk of ranking above the 85th percentile of total cholesterol, β-lipoprotein cholesterol (LDL), systolic blood pressure, weight and height distributions (specifically, 56%, 80%, 66%, 61%, 99% greater)'. These authors found no significant associations of blood type with α-lipoprotein (HDL). In transfusion science, fresh frozen plasma is collected for the preparation of the clotting factor Factor VIII. Blood group A plasma has been preferentially used for the production of this factor since it has been recognised that the activity of this factor is higher in group A plasma than in group O. Since Factor VIII is known to be heavily glycosylated there is an obvious question to be answered: does Factor VIII carry blood group ABH determinants and, if so, how does the subtle change in glycosylation affect factor activity?

Haemagglutination studies have suggested that blood group ABH and Lewis antigens are present on Factor VIII and von Willebrands factor [186]. Further characterization of von Willebrands factor and α-2-macroglobulin has shown that these two proteins carry ABH antigens according to the blood type of the donor and that these determinants are carried on N-linked chains [187]. Lima and co-workers [188] assessed the effect of blood group on the activities of Factor VIII:c and K-PTT and showed that group Os had lower activities of Factor VIII and higher levels of K-PTT than did group As. In the same year, Sweeney and Hoernig [189] demonstrated that vWF:Ag and Risocetin (RCo; high molecular weight multimers of vWF) were lower in the plasma of group Os and noted that a similar but more modest effect was seen in platelets. This work was verified by a study by Shima and colleagues [190] in 1995. These authors investigated levels of von Willebrands factor and specifically RCo and Botrocetin (BCo; mixed complexes of vWF) and demonstrated that all were affected by blood type. Heterozygotes carrying

**Table 9.** Blood group antigen changes and disease prognosis.

| *Cancer* | *Blood group change* | *Prognosis* | *Reference* |
|---|---|---|---|
| Oral squamous cell | Loss of H | High grade malignancy | [151] |
| Non-small cell lung | Expression of A | Increased survival | [146] |
| Prostate | Loss of ABH | Increased malignancy | [176] |
| Breast | Loss of $Le^b$ | Increased malignancy and invasiveness | [148] |
| Head and neck | Loss of ABH | Increased malignancy and invasiveness | [155] |
| Sarcoma | Re-expression of A or B | Cure, successful therapy | [179] |

the O gene had lower levels of these antigens than did homozygous As, Bs or heterozygous ABs. The lowest levels were found in group Os. Koster and his colleagues [191] demonstrated that blood group, vWF concentration and Factor VIII concentrations were all related to the risk of developing deep vein thrombosis. The risk increased with increasing vWF or Factor VIII and was higher in non-O blood groups than in group Os. Interestingly, the effect of oral contraceptives on anti-thrombin III levels is also modulated by blood group, strongly suggesting that this protein is glycosylated and that the glycosylation affects activity [192].

It has been suggested that terminal glycosylation either affects the conformation and/or binding properties of proteins or that it results in mislocalization of the proteins within the cell. As yet, it is unclear why so many of the proteins of the clotting cascade are elevated in group A individuals but it is not a 'quantum leap' to suggest that increased activity of clotting agents may contribute with other factors in triggering ischaemic heart disease.

Associations of blood type with other diseases have been reported for a range of disorders varying from coeliac disease to Achilles' tendon rupture [196]. A range of essentially auto-immune diseases, for example coeliac disease and NIDDM, are more common in non-secretors [193]. In capsular glaucoma formation, there is no association between blood group and pseudo exfoliation syndrome which precedes the full-blown disease, but once a group $A_1$ individual has this syndrome they are seven times more likely to develop capsular glaucoma [195] than any other blood type (Table 10).

## Proteins carrying blood group antigens: the effect on protein activity

A large number of proteins are known to carry blood group antigens, these range from gut hydrolases to clotting factors and glycosyltransferases themselves. For a number of these proteins there is no noted association between blood type and activity (Table 11). However, whether the appropriate experiments have been carried out is unclear. There are, however, a number of examples where the protein appears to be significantly changed by the addition of single sugar moieties (Table 12). EGFR has been discussed earlier in the context of cancer and clotting factors have been referred to earlier with respect to their modulation in individuals of different blood types and the possible association with ischaemic heart disease.

It has been known for 30 years that alkaline phosphatase levels are affected by blood type. Bamford and co-workers [207] and Arfors and colleagues [208] showed that the serum level of this enzyme is highly correlated with ABO type and secretor status. The highest levels are found in O and B non-secretors and much lower levels are apparent in the sera of all group As and secretors. This led to the suggestion that the terminal glycosylation affects the half-life of the circulating enzyme and that the proteins are cleared at different rates by carbohydrate binding proteins. In contrast the acid phosphatases do not appear to be affected by glycosylation [205].

It is clear that terminal glycosylation can have a profound effect on the activity of certain proteins. Whether this glycosylation itself is responsible for changes in conformation or binding properties of the protein is not clear. It is possible that there are specific endogenous lectins which bind differentially glycosylated proteins effectively reducing the levels of a protein when a specific terminal sugar is expressed. However, this would not explain the different effect terminal glycosylation appears to have on different proteins. We are only ever looking at the end result of a complex system involving numerous enzymes and proteins, the expression of which is regulated by molecules which interact with the DNA. If such molecules were affected by glycosylation we may be seeing the results of over- or under-expression of certain proteins whose function is regulated by these molecules: an indirect effect on translation rather than an intrinsic change in the activity of the protein.

**Table 10.** Association of blood group with disease.

| *Disease* | *Blood group affected* | *Reference* |
|---|---|---|
| Coeliac disease | Non-secretors | [193] |
| Gastro-duodenal ulcers | O non-secretors | [194] |
| Ankylosing spondylitis | Non-secretors | [193] |
| Graves disease | Non-secretors | [193] |
| Non-insulin dependent diabetes | Non-secretors | [193] |
| Capsular glaucoma | $A_1$ | [195] |
| Ruptured Achilles tendon | O | [196] |
| Heart disease | A | [182–185] |
| Parathyroid water clear hyperplasia | O | [197] |

**Table 11.** Proteins carrying ABH antigens in which no association of activity with blood group is seen.

| *Protein* | *Reference* |
|---|---|
| Intestinal hydrolases | [198–200] |
| *β*-galactosyltransferase | [201] |
| *α*-*N*-acetylgalactosaminyltransferase (*A*) | [202] |
| RBC Band 3, 4, 5 and PAS 1 and 2 | [203] |
| Sucrase isomaltase | [204] |
| Acid phosphatase | [205] |
| Complement receptor regulatory protein | [206] |

**Table 12.** Proteins whose activity is affected by blood type.

| *Protein* | *Blood type* | *Effect* | *Reference* |
|---|---|---|---|
| Epidermal growth factor receptor | A vs O | Change in affinity and factor binding | [163, 164] |
| *H* transferase | A | Increased activity vs O | [202] |
| vonWillebrands factor | A | Increased activity vs O | [189] |
| Factor VIII | A | Increased activity vs O | [188] |
| Alkaline phosphatase | O and B | Increased activity vs all As and non-secretors | [207–212] |

## The evidence so far

This paper has focused on data collection and the associations described between disease and blood groups. In many cases the evidence for these associations has been provided by statistical analyses. Some of the studies have been well-performed whereas others have not investigated all parameters fully. For example, most do not consider the *ABO* genotype but relate their findings simply to the phenotypic data. In the case of the association of plague with blood group O [115], it was difficult to explain the high frequency of the O phenotype in populations in which plague had been endemic until it was appreciated that many As and Bs are heterozygotes and carry the *O* allele silently. Thus the *O* allele was maintained in the heterozygote population. In many early studies the secretor status and Lewis phenotypes were not considered.

In other cases, the tissue distribution of the antigens has not always been fully appreciated. For example a blood group A non-secretor will produce mucins devoid of A antigen but a small number of the cells of the gut which are not under the control of the secretor gene may well express A. Thus, any micro-organism which is bound by blood group A antigen may be trapped in the mucin and removed in secretors whereas in non-secretors the organism may survive the protective mucin and then 'latch onto' A antigen in the underlying non-secretory cells [213].

A systematic study must be undertaken prove or refute the statistical data utilizing a more scientific approach. We must determine for example, whether micro-organisms carry blood group antigens and if so do these really interact with natural antibodies allowing clearance of the organism by the immune system. We may also need to address the shape of the sugars carried on bacterial and viral surfaces and determine whether there is less obvious cross reactivity between bacterial and viral antigens and human antibodies and lectins. Molecular modelling [214–217] studies may provide answers to questions of similarity between cross-reacting but apparently dissimilar molecules. The specificity and nature of the adhesins and lectins should be reviewed in the light of the statistical data. How can we explain the results suggesting that *H. pylori* binds to the $Le^b$ antigen when this is not apparently consistent with the statistical data showing that gastric and duodenal ulcers are more commonly found in group O non-secretors [100–104]? We must also determine the fate of micro organisms following binding and the role played by protective proteins such as the mucins. There is obviously a pressing need to understand the data since the results may suggest a role for carbohydrates in therapy. If, for example *H. pylori* does bind specifically to $Le^b$ then we may be able to inhibit the binding by the addition, possibly to the diet, of carbohydrate molecules which would compete with the endogenous antigen.

In microbial and protozoan infections the role of glycosidases should not be overlooked. In the case of influenza virus, therapeutic molecules have been formulated which inhibit the neuraminidase activity vital for the pathogenicity of the organism. Such therapy may be appropriate for other infections where hydrolysis of carbohydrates plays a role in pathogenicity [218].

In the evidence presented, there does appear to be a distinct disadvantage to those carrying the blood group *A* gene since more As suffer from cancer [135, 166–171] and heart disease [182–185] than Os. Obviously we cannot change our blood groups although it has been said that the key to a long life is to choose your parents with care – a luxury we cannot indulge. So what can be done with this knowledge? In cancer for example, we have spent much time and resources on an exploration of the cell surface antigens, but it is clear that the cell comprises thousands of proteins the properties of some of which may be modulated by their terminal glycosylation. What are the effects of changing a cell expressing $Le^a$ into a cell expressing A, H and $Le^b$? The evidence suggests that growth factor receptors, oncogenic proteins and enzymes will all be affected changing the delicate balance within the cell. One must assume that individual cells from group As, Os, Bs and ABs have developed a *status quo*. Their well-being is guarded by a homoeostatic mechanism and the cells compensate to suit their environment. However, if a single cell turns off a gene inappropriately it will continue to be affected by messages from the surrounding cells which will be expressing proteins which are normally glycosylated. The receptors and products of the aberrant cell however may not respond to these

signals in the same way as the normal cell. Could this act as a trigger in cancer formation?

How can these questions be addressed when the answers can only be gained by investigating humans? It is necessary to realize that although animal models may give clues, their terminal glycosylation is different from humans and therefore they may not provide valid answers. It has been suggested that transgenic mice may be able to supply the answers; the 'Rosetta stone' in glycobiology [219]. It is hard to anticipate their immediate use in investigations of this type, but glycosylation is already being manipulated in the transgenic pigs destined for xenografting so such technology may be closer than we think. Investigations involving continuous cell lines are inappropriate since we know that cell lines frequently demonstrate abnormal glycosylation reflecting the undifferentiated status of the cells. Experiments involving non-transformed cells suffer from reproducibility problems since the life-span of the cells is so short. The true experimental model, man himself, will undoubtedly be reluctant to co-operate in our experiments. Questions such as 'what happens when I put intestinal cells into a normal human colon, emulating some of the changes seen in carcinogenesis?' may never be answered, unless of course some surgeon has already carried out such a procedure without realizing why glycobiologists could be interested. Maybe we should encourage the non-glycobiologist to read about our work and recognize that we all have common interests. Currently we can only evaluate the information we can gather and try to employ methods to explain our results.

The advent of molecular biology also offers us new tools to turn off genes using anti-sense oligonucleotides and to introduce novel genes into cells. Theoretically, a short anti-sense oligonucleotide which recognizes a unique area of the desired gene may be synthesized, chemically modified and stabilized and then introduced either into cells or whole animals. The oligonucleotide will then interact with mRNA, forming a double stranded molecule which cannot be translated. This approach has been used in cell culture and animal models and has been validated for use in the treatment of some human diseases. Such technology could allow us to address questions as to the effect of having genetically identical cells which differ solely in their blood group antigen repertoire growing together in culture. Workers have already tried this approach to corroborate studies showing that cells carrying A, H and I antigens vary in their susceptibility to immune cell recognition and cytotoxicity [172]. Such technology will allow us to reverse the changes in glycosylation in certain cancer cells and determine the effect.

Addition of genes is less straightforward and suffers from the lack of regulated control of DNA integration into cells. Theoretically targeted mutagenesis or homologous recombination could be used, but these methods are most useful in scenarios such as embryonic stem cell manipulation and the production of transgenic animals where a selection process can be applied to ensure the correct localization of the DNA. Incorrect localization of the gene product could severely affect the validity of the results.

The effect of glycosylation on the activities of proteins is certainly not clear. Studies could be carried out to establish the specific activity of proteins of interest purified from individuals of different blood groups or by quantitation of mRNA specific for the proteins of interest. The former could be a laborious process since some of these proteins are present in very small amounts and are known to be labile. The latter is also difficult but methods are available to quantitate mRNA. The real problem lies in the accessibility of the organs manufacturing the proteins. In the case of the analysis of mRNA for Factor VIII we would need to take liver biopsies from a range of 'normal' individuals. Such a procedure may be construed as unethical. Additionally, Factor VIII has more than 20 potential glycosylation sites. Are they all important? Indeed are they homogeneous with respect to glycosylation? Factor VIII poses such difficult problems that it will not be the most obvious 'model' protein to analyse. Each of the protein factors implicated in heart disease could be analysed using similar procedures. If A antigen affects the proteins directly we would need to determine how the addition of a single sugar, or, if the factors from A and B bloods differ, the addition of a single N-acetyl-amino group to the end of a sugar chain could change the activities of a molecule such as Factor VIII.

## The future – the crystal ball

The advances science has made in the last 20 years were certainly not predicted. The development of a single simple technique, PCR, has revolutionized molecular biology. We can now analyse a single egg for genetic defects, clone genes rapidly, introduce mutations into genes and produce proteins of choice almost at will. Who knows whether a new tool for glycosylation analysis is just around the corner? Molecular biology has revolutionized the studies of protein biosynthesis and regulation and will surely play a large part in the studies of the effects of glycosylation, allowing us to manipulate the nature of the sugars expressed on individual proteins. It is a sobering thought that the single feature of proteins which still makes the production of recombinants a problem is glycosylation.

The new electronic conferencing harnesses advances in computer technology to facilitate interactions and allow us to share information: this is a key to the future. We must look outside our own fields, encourage non-glycobiologists to ponder whether they have data or materials to offer which would help us all solve problems. For example, subtle changes in antigens on the red cell can provide a wealth of information. Two children who presented as paraBombay, lacking H, $Le^b$, $Le^a$ and $Le^x$ antigens, were shown to possess all of the relevant fucosyltransferases for antigen synthesis [220]. It was suggested that they had a general defect in fucosylation. These children also suffered from a rare

condition which affected their lymphocytes. It was subsequently shown by Frydman and his colleagues [221] how a simple red cell abnormality in a patient with an inherited disease can be used to strengthen the evidence for the role of fucosylated antigens in lymphocyte homing. Yamamoto and co-workers [89, 222, 223] have already studied a number of the 'more common' rare sub-groups but as yet have not correlated enzyme characteristics with gene mutations. A collaboration of more than twenty years with transfusion centres world-wide has facilitated the analysis of the properties of the glycosyltransferases from more than twenty distinct sub-groups of ABO [202]. Analysis of the mutations which bring about these changes in the enzyme will give clues to the importance of specific amino acids in substrate binding, catalysis, enzyme stabilization and enzyme secretion. The results will provide information which will be most useful to both those studying glycosylation and to those producing recombinant glycosyltransferases.

Computer modelling studies and virtual reality modelling are useful, but currently we find it difficult to model proteins with their respective sugar moieties. The technology allows us only to examine individual glycoproteins and does not reflect the *in vivo* situation in which the glycoproteins are affected by molecules in the environment and other protein, glycoprotein and sugar molecules within the cell. The future of glycobiology must now be aligned with cell biology. We need to gain an understanding of why proteins are glycosylated and how glycosylation affects individual cells and the whole organism. Glycosylation is arguably the most important modification made to a protein: an understanding of the mechanisms and processes involved is of paramount importance. We have all heard the molecular biologist talk of DNA as the molecule of this decade: sugars should be the molecules of the next.

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Received 19 March 1996, accepted 4 June 1996