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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The Distribution and Characteristics of Ly-6 in Normal and Diseased Murine Kidney and of its Analogue, CD59 (Protectin), in Normal and Diseased Human Kidney

by

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ABSTRACT

Ly-6 is a murine multigene family of cell surface glycosyl-phosphatidylinositol anchored proteins. Ly-6 molecules are widely expressed on lymphoid tissue where they are interferon (IFN) regulated and may participate in lymphocyte activation. Renal and other extralymphoid expression of Ly-6 has been reported but never characterised in detail.

In this study we examined the characteristics of renal Ly-6 expression using monoclonal antibodies and cDNA probes. We also studied expression of CD59, a human analogue of Ly-6, in normal and diseased human kidney.

Renal Ly-6 expression was high relative to that in other organs with Ly-6A/E being expressed on vascular endothelium and tubular epithelium, especially in the distal nephron. Ly-6A and E showed allele specific variation in their relative expression between renal cortex and medulla. Ly-6C was not detected in kidney by antibody staining but was by oligonucleotide probing and ribonuclease protection assay. Renal cells expressing Ly-6 RNA were radiation resistant suggesting that tubular expression reflects de novo synthesis and not protein resorption. Ly-6 RNA was upregulated within 6 hours of IFN-γ administration and the increase was most evident on the luminal aspect of proximal tubular epithelium. Similar renal Ly-6 upregulation was noted in murine lupus and in mercuric chloride nephropathy,

diseases in which IFN-γ plays a role, but not in apoferritin immune complex nephritis. Ly-6A/E was also detected on endothelium and the luminal aspect of ductular epithelium in other organs.

CD59 was found on vascular endothelium, the luminal aspect of tubular epithelium and glomerular endothelium and mesangium in 'normal' kidneys from transplant donors and patients with minimal change disease. Expression did not change in acute tubular necrosis or glomerulonephritis but upregulation of tubular expression was seen in acute interstitial nephritis and acute graft rejection.

Ly-6 is prominently expressed on epithelium and endothelium in kidney where it is IFN-γ regulated and shows locus and allele specific variations in expression. CD59 shows similar distribution in human kidney and both Ly-6 and CD59 are upregulated in particular renal diseases. Hypotheses concerning the functions of Ly-6 and CD59 should take into account their marked renal and other extralymphoid expression.

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ABBREVIATIONS

AR - acute graft rejection

AIN - acute interstitial nephritis

ATN - acute tubular necrosis

cDNA - complementary DNA

DAF - decay accelerating factor

DNA - deoxyribonucleic acid

GN - glomerulonephritis

GPD - glyceraldehyde phosphate dehydrogenase

GPI - glycosyl-phosphatidylinositol

HAF - horse apoferritin

HRF - homologous restriction factor

ICAM-1 - intercellular adhesion molecule-1

IFN - interferon

i/p - intraperitoneal

kb - kilobases

lpr - lymphoproliferative

LPS - lipopolysaccharide

mAb - monoclonal antibody

MAC - membrane attack complex

MACIF - membrane attack complex inhibiting factor

MHC - major histocompatibility complex

MIRL - membrane inhibitor of reactive lysis

PBS - phosphate buffered saline

PNH - paroxysmal nocturnal haemoglobinuria

poly IC - polyinosinic-polycytidylic acid

RNA - ribonucleic acid

RNase - ribonuclease

SLE - systemic lupus erythematosus

TAP - T cell activating protein

TCR - T cell receptor

THGP - Tamm Horsfall glycoprotein

CHAPTER ONE

INTRODUCTION - LITERATURE REVIEW

PART 1: Ly-6

Ly-6 is a multigene family of murine polymorphic cell membrane proteins that has received substantial attention in recent years[1,2]. It was first defined in 1977 by McKenzie and co-workers using alloantibodies against murine lymphocytes[3]. In retrospect many alloantisera against these highly antigenic proteins had been described previously under different names, including Ala-1, DAG and Ly-8[4-7]. Two principal haplotypes were subsequently identified and named as Ly-6.1 (Ly-6^a) and Ly-6.2 (Ly-6^b) and a recombinant strain, C3H.B6-Ly-6^b, was derived[7,8].

SEROLOGY AND NOMENCLATURE

The first monoclonal antibodies (mAbs) against Ly-6 antigens were raised and reported by three separate groups in 1980[9-11]. These antibodies detected antigens with identical strain but distinct tissue distributions, suggesting that there was close genetic linkage but not identity and it consequently became apparent that Ly-6 comprised a complex family of antigens. These initial mAbs were all

reactive with Ly-6.2 haplotype specificities but, subsequently, Ly-6.1 mAbs were also raised and a nomenclature for the Ly-6 antigens was developed by Kimura and Hammerling[12]. This nomenclature designated 5 specificities, Ly-6A to E, which in turn were identified as 1 or 2, depending on which haplotype they were associated with. Thus, there was Ly-6A.2, identified by Kimura's original S8.106 mAb, and there was Ly-6C.2, identified by Takei's H9/25 mAb, and there was Ly-6E.1, identified by Kimura's SK70.94 mAb[9,10].

Unfortunately problems have arisen with this nomenclature which have made the Ly-6 field difficult to follow for the non enthusiast! Firstly, for no apparent reason, Eckhardt and Herzenberg's ThB specificity received no Ly-6 designation even though it was clearly a member of the family[11]. Secondly, some investigators continued to assign new and different names to Ly-6 molecules so that, for example, Ly-6A.2 became widely known as T cell activating protein (TAP) for reasons that will soon be apparent. Finally, Ly-6A.2 and Ly-6E.1, which were originally thought to be distinct antigens, were subsequently found to be only alternate allelic gene products[13] and so should be renamed Ly-6A.2 and A.1 respectively but, in the absence of a nomenclature committee, this has not actually happened[14]. Thus the original nomenclature persists despite these imperfections.

BIOCHEMICAL CHARACTERISATION

The generation of these Ly-6 mAbs facilitated the biochemical characterisation of the various Ly-6 proteins. Ly-6A.2, E.1, B, C and ThB have all been immunoprecipitated and studied using electrophoresis and other techniques[10,16-17]. All are relatively small proteins, having molecular weights of 10 to 18 kd and all are members of the structurally and functionally diverse 'family' of molecules linked to the cell membrane by a glycosyl-phosphatidylinositol (GPI) anchor[13,17-20]. Ly-6A.2 and E.1 are the best characterised and have been shown to be glycoproteins containing O-linked but not N-linked polysaccharides and both have decreased electrophoretic mobility on reduction, indicating the presence of disulphide bonds[134].

CLONING OF Ly-6 GENES

N-terminal amino acid sequence data from Ly-6 proteins was used to clone a number of Ly-6 genes between 1986 and 1988[21-24]. Ly-6A.2 and E.1 cDNA's were found to contain a 402 nucleotide open reading frame and the corresponding amino acid sequence was found to comprise a hydrophobic leader sequence of 26 residues and a translated protein of 108 residues, the last 29 of which are cleaved off when the GPI anchor is attached at the carboxy terminal. Ly-6C.1 and C.2 were subsequently cloned and sequenced and their amino acid composition

was also deduced. Comparison between the predicted protein sequences of Ly-6A.2 and E.1 showed that they differed by only 2 amino acids, corresponding to 3 nucleotide differences, and that one of these amino acid differences was at the carboxy-terminal and so would not be represented in the mature protein[21,22]. Similarly, Ly-6C.1 and C.2 differed at only 2 amino acids, corresponding to 2 nucleotide differences, and again only one amino acid difference is found between the mature proteins[23,24]. Comparison of Ly-6C.1/C.2 and E.1/A.2 showed 83% and 50% homology at the nucleotide and amino acid level, respectively. In all 4 proteins, there were 10 cysteine residues with highly conserved positions and maximum homology was found in the leader sequence and amino-terminus and in the hydrophobic carboxy-terminus. None appeared to have transmembrane or cytoplasmic domains, consistent with the evidence for GPI anchoring of the mature protein.

Genomic southern blotting showed that Ly-6 probes hybridized to as many as 30 distinct genes, suggesting that Ly-6 may be a large multigene family[25]. In situ hybridization localized the genes to chromosome 15, band E, a region associated with the c-sis and c-myc protooncogenes, as well as with alterations related to lymphoid malignancies[25]. The Ly-6A.2 and C.1 genomic structures have been elucidated and both comprise 4 exons are approximately 4 kb long and have 78% nucleotide similarity suggesting that they arose by gene duplication[24,26].

Initially no homology was detected between Ly-6 and previously characterised genes but, subsequently, related sequences were identified in a protein in squid brain[27], Ly-6 analogues in sheep and rat[28,29] and in 2 human proteins, CD59 and the urokinase receptor[30,31]. This has given rise to the concept of the 'Ly-6 superfamily'[31]. All this suggests that this gene structure may be phylogenetically old and well conserved.

LYMPHOID DISTRIBUTION AND FUNCTION

Studies with mAbs indicated that the various Ly-6 proteins had distinct distributions on lymphoid and other haematopoietic tissues[9,13,32,33]. For example, Ly-6A.2 was on 50-70% of peripheral lymphocytes and all T and B cell blasts and on some thymocytes while Ly-6C.2 was on a large subpopulation of marrow cells and on about 50% of CD8 positive peripheral lymphocytes but not on thymocytes. More surprisingly, Ly-6E.1 differs from its allelic variant Ly-6A.2 in having markedly lower levels of expression on peripheral lymhocytes[34,35]. Therefore, the Ly-6 proteins show not only locus specific but also allele specific variations in tissue expression. This latter finding is somewhat unusual.

In 1986 the first clues to a function for Ly-6 proteins became apparent when Rock et al reported that an Ly-6A.2 mAb could stimulate T cell activation, as defined by proliferation and IL-2 production, in both hybridomas and normal T

cells[36]. It soon became clear that Ly-6C and E mAbs had the same stimulatory capacity although, in most cases, cross-linking by anti-immunoglobulin or by Fc-receptor bearing accessory cells was required for activation[37,38]. Ly-6 mediated T cell activation does not require a rise in intracellular free calcium but does depend on internalisation of the antibody bound Ly-6[2]. Thy-1 mAb induced T cell activation also requires similar internalisation and, as both Thy-1 and Ly-6 are GPI anchored proteins, this may be a general feature of activation mediated by such proteins[2]. Ly-6 has thus been included in the ever expanding list of accessory molecules involved in so called 'alternative pathways of T cell activation'[39], many of which have more recently been referred to as adhesion molecules[40].

Experiments using normal and mutant T cell receptor (TCR) negative T cell hybridomas have shown that Ly-6 mediated activation cannot occur in the absence of TCR but is restored by appropriate transfections with the missing receptor chains[41]. Conversely, Ly-6 negative mutant T cell lines show a 1000-fold reduction in IL-2 response to anti-TCR induced activation and, similarly, Ly-6 antisense oligonucleotides completely inhibit or greatly reduce activation of both hybridoma and antigen primed T cells in response to both CD3 mAb and concanavilin A[42,43]. Thus, it appears that normal T cell activation may require both an intact TCR-CD3 complex and normal Ly-6 expression. These experiments greatly increased interest in Ly-6 proteins and led to the widespread belief that their function was as accessory molecules in T cell, and also in B-cell, activation[44]. More recently, some

doubt has arisen about the physiological significance of this function in the light of increasing evidence that mAbs to any GPI anchored protein, including such unlikely candidates as 5' nucleotidase and decay accelerating factor (DAF), can also mediate T cell activation *in vitro*[45,46]. This property may be related to the actual anchor rather than to the protein itself and so may not represent the full molecule's real physiological function.

INTERFERON RESPONSIVENESS

Cytokine responsiveness of Ly-6 expression was first reported by Dumont et al who showed in 1986 that both interferon (IFN)-α/β and γ increased Ly-6 A.2 and E.1 and, to a lesser degree, C expression by T cells[47,48]. Similar results were reported for IFN-γ and Ly-6C on endothelium and macrophages and for Ly-6A/E and/or C in a variety of cell lines treated with either IFN-α/β or γ[49]. In view of these findings it is not surprising that an IFN consensus sequence, similar to but distinct from that related to the MHC genes, has been identified upstream of the Ly-6C gene[24]. Presumably, a related sequence must also exist for the Ly-6A/E genes.

EXTRALYMPHOID Ly-6 EXPRESSION

Extralymphoid and in particular, renal expression of Ly-6 was first noted over

a decade ago by McKenzie et al[3] in the original paper defining Ly-6 and, a year later, by Halloran et al[50]. In both cases the methodology involved tissue absorption of antisera. Subsequently, using tissue absorption of mAbs, Kimura et al detected Ly-6A.2 in kidney, brain and liver[10], and Hogarth detected Ly-6C in kidney and liver[33]. In 1989 there were the first reports of nonlymphoid tissue sections being stained with an Ly-6 mAb, when van de Rijn et al, who were investigating a murine haematopoietic stem cell marker called Sca-1, discovered that it was identical to Ly-6A.2[51]. Using an indirect immunoperoxidase technique, they detected Sca-1/Ly-6A.2 on endothelium of heart, brain and liver and on endothelium and tubules of kidney, but no detailed characterisation of distribution was reported. Once Ly-6 cDNAs were available, both Reiser and LeClair investigated tissue distribution, using Northern blotting, and showed RNA expression, in approximate descending order of magnitude, in kidney, spleen, thymus, lung, heart, liver and brain[21,22]. However, as of 1992, no detailed characterisation of the distribution and properties of extralymphoid Ly-6 had been reported. Extralymphoid expression may be of particular importance because its existence, of course, raises the possibility of alternative functions other than mediation of lymphocyte activation for Ly-6.

Ly-6 IN DISEASE

The role of Ly-6 in disease has received little attention. Ly-6C expression

on lymphocytes has been shown to be absent in both the NZB model of murine SLE and in the NOD model of murine autoimmune diabetes[52]. More recently, the latter observation in NOD mice has been confirmed by Herold et al who also showed that the missing Ly-6C expression can be restored *in vitro* by stimulation of lymphocytes with anti-CD3 and that Ly-6C positive T cells are present in the inflamed islets of these animals[53]. These findings suggest that Ly-6C is acting as a marker of activated T cells in this condition. There has been no investigation of renal Ly-6 expression in disease.

RECENT DEVELOPMENTS

Interest in Ly-6 has heightened in the light of a number of recent discoveries. Firstly, the aforementioned discovery by Weissman's group at Stanford that Ly-6A.2 is a marker of the murine pluripotential haematopoietic stem cell has been of enormous interest to the haematologic community[51]. Secondly, the discovery of a possible human analogue, CD59, by Stefanova et al[30] and its subsequent cloning and identification by a number of separate groups as a homologous complement restriction factor[54-56], the deficiency of which may cause the disease, paroxysmal nocturnal haemoglobinuria (PNH), has greatly increased the potential relevance of Ly-6 to human physiology and pathology. Thirdly, the discovery of sheep and rat Ly-6 equivalents and of a squid homologue[27-29], and of homology between Ly-6 and the human urokinase plasminogen activator

receptor have raised the possibility of there being an extensive Ly-6 superfamily extending far back into phylogeny[31]. Finally, there has recently been a great interest in the biology of molecules with GPI anchors with particular attention to their functions and distributions and Ly-6 has provided a model for some of these studies[18-20].

PART 2: CD59

CD59, the first human homologue of Ly-6 to be identified, was originally described by Stefanova in 1989 during the course of a project which involved preparing a series of mAbs against previously undefined human leucocyte surface antigens[30]. One such mAb, known as MEM-43, was found to recognise an antigen that was expressed on leucocytes and erythrocytes and that turned out to be a GPI anchored glycoprotein with a molecular weight of 18-25 kd. N-terminal amino acid sequencing of the protein revealed a strong homology to Ly-6. This protein was then christened CD59 at the 1989 Fourth International Conference on Human Leucocyte Differentiation Antigens[57]. Amazingly, during 1989 identification of this same human protein was reported by 6 other separate groups! Davies working with Lachmann and Waldmann in Cambridge and using a mAb called YTH 53.1, purified the protein and cloned and sequenced the cDNA[54]. They confirmed that it was a GPI linked 20 kd glycoprotein and found 26% amino acid sequence homology with Ly-6E.1. Moreover, they reported that this protein inhibited the

emerged since 1989, dealing with CD59 as a complement restriction factor and as an adhesion and activation molecule, respectively.

CD59 AND COMPLEMENT

It is now apparent from studies by Meri and by Rollins that CD59 acts to restrict complement lysis by inhibiting the C5b-8 catalysed insertion of C9 into the lipid bi-layer of the target cell membrane[63,64]. The initial loose binding of C9 to the C5b-8 complex in the cell membrane occurs normally in the presence of CD59. However, the subsequent tight binding of C9 to a higher affinity site on C5b-8 and the consequent unfolding of C9, which allows recruitment of other C9 molecules to form the actual cytolytic C5b-C8-polyC9 complex, is blocked by an interaction of CD59 with this high affinity site on C5b-8. As a result, CD59 protects against cytolysis and so Meri et al christened it 'Protectin'[63].

Clinical relevance for CD59 became apparent when a case report in the New England Journal of Medicine in 1990 described a patient with PNH, a haemolytic anemia which had previously been thought to be related to the absence of all proteins with GPI anchors from the surface of erythrocytes in affected individuals[65]. It had been thought that the most critical deficiency in these patients was that their red cells lacked DAF which is another GPI anchored complement inhibiting protein[66]. However in this case report, the patient had normal amounts

membrane attack complex (MAC) of complement and that the mAb was able to enhance lysis of human erythrocytes and lymphocytes by homologous complement[54]. Sawada cloned the same gene and found 35 and 31% homology with Ly-6C and A respectively and also that the characteristic Ly-6 alignment of cysteine residues was conserved in CD59[58]. Both of these studies noted that, on genomic southern blotting, CD59 appeared to be represented by a simple gene, in contrast to the multigene Ly-6 complex.

Meanwhile, Holguin et al isolated a protein capable of inhibiting complement lysis of erythrocytes and called it 'membrane inhibitor of reactive lysis (MIRL)'[59] and Okada discovered a protein with the same function and called it 'homologous restriction factor-20 (HRF-20)', in view of its 20 kd molecular weight and in contrast to the previously described HRF-68[60]. Sugita had actually discovered the same protein a year earlier and called it 'MAC inhibiting factor (MACIF)' or P-18[61]. MIRL, HRF-20 and MACIF all turned out to be identical to CD59 so that, in retrospect, Sugita had actually discovered the protein first but had not sequenced it and so did not detect the homology to Ly-6. Finally, Groux et al in Paris described an adhesion molecule called H-19 which they discovered to be present on erythrocyte, monocyte and lymphocyte cell surfaces, to be involved in rosette formation and to be required for T cell activation[62]. H-19 was also the same as CD59 and this raised the possibility of the protein having a function other than complement regulation. Consequently, two separate sets of literature have

of DAF and of other GPI anchored proteins but was completely deficient in CD59 and had typical PNH. This was taken to indicate that the cause of PNH is deficiency of CD59 and not of DAF nor of other GPI anchored proteins.

CD59, AN ADHESION AND ACTIVATION MOLECULE

After Groux's initial report that CD59 was involved in adhesion events and in T cell activation[62], these apparent functions of CD59 received less attention until, in 1991, Korty from Shevach's laboratory at NIH, reported that an anti-CD59 mAb could, in the presence of appropriate co-stimulators, activate T cells[67]. Activation was measured by increased intracytoplasmic calcium, inositol phosphate turnover, IL-2 production and cell proliferation. CD59 mediated activation was dependent on co-expression of TCR/CD3 but the converse was not the case. These findings are reminiscent of those for Ly-6 mediated T cell activation[36] but these observations must be qualified by reiterating that mAbs to every GPI linked surface protein appear to be capable of activating T cells, so that the physiological significance of these findings is unclear[68].

IS CD59 THE HUMAN Ly-6?

Homology between Ly-6 and CD59 is approximately 25% at the amino acid level and 35-40% at the nucleotide level. There is also significant sequence

alignment between these proteins, especially with regard to cysteine residues. In addition, there are 3 areas of particular homology, one at each terminus and one at the proposed site for proteolytic cleavage and subsequent attachment of the GPI anchor[54,58,60]. However, in spite of these quite impressive interspecies homologies, there remain reasons to doubt that CD59 is the human equivalent of Ly-6.

Firstly, Ly-6 is a multigene family while CD59 has repeatedly been shown to be encoded for by a single gene[58,69]. Secondly, Ly-6 is clearly IFN inducible but studies to date have not found this to be the case for CD59[69]. Thirdly, CD59 has two potential N-glycosylation sites while Ly-6 has none[69]. Finally, there are notable differences in the tissue distribution of Ly-6 compared to that of CD59[69,70]. In particular, erythrocytes do not express detectable Ly-6, whereas they represent a critical site of expression for CD59. Thus, a reasonable conclusion might be that human CD59 is a structural homologue but not an exact equivalent of murine Ly-6.

TISSUE DISTRIBUTION OF CD59

In one of the initial descriptions of CD59, Sawada et al, using a cDNA probe and northern blotting, found RNA transcripts in lung, placenta, tonsil, pancreas and peripheral lymphocytes but not in kidney or brain[58]. However, in subsequent studies, Okada's group, using the IF5 CD59 mAb, detected CD59 on vascular endothelium and on epithelial tissue, including renal tubules, bronchi, salivary ducts

and ependyma[71]. Lachmann's group, using YTH53.1, a different mAb, confirmed these findings in a more detailed study[70,72]. They detected CD59 on cutaneous epidermis, vascular endothelium, biliary, pancreatic and salivary ducts, neural Schwann sheath, placental syncytiotrophoblast and pulmonary bronchi, as well as on renal distal tubules, collecting ducts and glomerular endothelium. The similarity to tissue distribution of DAF was noted[72,73]. There is little information on alterations in CD59 expression in disease, other than its apparent absence from erythrocytes in individuals with PNH. One study from Okada's group suggested that glomerular CD59 expression was enhanced in lupus nephritis compared to that in normal kidneys and in other glomerular diseases[74]. More investigation is required in this area, particularly in diseases where complement mediated injury is likely to be playing a role.

Part 3: PURPOSE OF THIS STUDY:

The purpose of this body of work was to characterise the distribution and properties of Ly-6 in normal and diseased murine kidney and in other extralymphoid organs. The essential questions being asked were: (1) what was the relative amount of Ly-6 in kidney relative to that in other lymphoid and non lymphoid organs? (2) what was the actual distribution of Ly-6 within the kidney and which Ly-6 proteins were responsible? (3) was renal Ly-6 responsive to IFN or IFN inducing agents and, if so, what were the characteristics of the response? (4) was

renal Ly-6 expression affected by radiation? (5) how was renal Ly-6 expression affected by the presence of renal disease? (6) what was the distribution of Ly-6 within other nonlymphoid tissues and was it altered by IFN or by IFN inducers?

With the discovery of CD59, the scope of the work was extended to study the expression of this protein in normal and diseased human kidneys in order to ask: (1) what is the normal CD59 distribution in kidney and how does it compare to that of Ly-6? (2) how is CD59 expression altered in a variety of renal diseases, both glomerular and interstitial?

Finally, the answers to all these questions were considered together in an attempt to draw some conclusions about the nature and function of the renal expression of these fascinating and mysterious proteins.

CHAPTER TWO

MATERIALS AND METHODS

MICE

BALB/c (H-2^d), CAF1 [(BALB/c x A)F1] (H-$2^{d/a}$) and CBA/J (H-2^k) mice, which express the Ly-6.1 haplotype, were housed at our animal colony. A.D2-Ly-6b (H-2^k) mice, which are congenic with strain A/J at the Ly-6 locus and express the Ly-6.2 haplotype, were produced, bred and housed at our colony. MRL lpr/lpr mice which carry the autosomal recessive lymphoproliferative (lpr) mutation[75] and MRL +/+ mice which do not were also housed at our animal colony. All mice were between 6 and 14 weeks of age during the experiments.

ANTIBODIES

YE3, a rat IgG mAb to an Ly-6 A\E determinant, was provided by Dr Fumio Takei (B.C. Cancer Research Centre, Vancouver)[76]. Ly-6A.2 and E.1 are thought to be alleles at the same locus with the former being expressed by Ly-6.2 and the latter by Ly-6.1 murine strains respectively[1,2]. 34-11-3 and 34-2-11, murine IgG mAbs to Ly-6A.2 and C.2 respectively, were provided by Dr David Sachs[77]. M1, a rat anti murine class I IgG mAb, and H-2D and H-2K, murine anti murine class I

IgG mAbs, were obtained from American Type Culture Collection (ATCC), Bethesda, MD.

INTERFERON-γ INDUCTION

This was done both by directly administering recombinant mouse IFN-γ 100,000 units intraperitoneally (i/p) (supplied by Dr Peter Van Der Meide, Primate Centre TNO, Rijswijk, Netherlands)[78]. In addition, the effect of agents known to induce IFN production *in vivo* in the context of different immune responses was investigated. The latter were: (1) S. Minnesota lipopolysaccharide (LPS) (Sigma Chemical Co., St Louis MO) which induces both IFN-α/ß and IFN-γ in a T cell independent manner[79]; (2) polyinosinic-polycytidylic acid (poly IC) (Sigma Chemical Co., St Louis MO) which induces mainly IFN-α/ß in a manner resembling viral infection[80]; (3) oxazolone (4 ethoxymethylene-2-phenyl-2-oxazolin-5-one) (Sigma Chemical Co.) which induces IFN-γ in a delayed type hypersensitivity-like response[81]; (4) DBA/2 mastocytoma P815 cells, obtained from ATCC, and maintained by weekly passage in female DBA/2 mice in ascitic form and which also induce IFN-γ in an allogeneic rejection response[80]. Control mice were treated with topical acetone and i/p phosphate buffered saline (PBS). These agents were administered and tissues harvested according to the protocols outlined in Table 1. Tissues harvested included liver, spleen, pancreas, heart, lung and salivary gland, as well as kidney.

RADIATION

Female CBA/J mice were subjected to γ radiation doses of 500, 750, 1000, 1500 and 2000 rads on day one and sacrificed on day 5. Kidneys were harvested for RNA extraction.

MURINE MODELS OF NEPHRITIS

Three murine models of nephritis were investigated:

1. *Murine lupus:* MRL lpr/lpr and control MRL +/+ mice were sacrificed at 6 and 17 weeks and kidneys were harvested for frozen sectioning and immunoperoxidase staining and for RNA extraction.

2. *Mercuric chloride nephropathy:* CBA/J mice were administered mercuric chloride (Sigma Chemical Co.) at doses of 1.6., 2.0., and 3.2 mgs per kg body weight three times weekly as previously described[81]. Mercuric chloride was injected i/p in a 0.4% solution in 0.9% sterile saline. Control mice were injected i/p with 0.9% sterile saline. Both treated and control mice were sacrificed at one week as this is when induction of other IFN regulated surface proteins has been shown to be maximal[82]. Kidneys were then harvested for immunoperoxidase staining and RNA extraction.

3. *Horse apoferritin (HAF) - immune complex nephritis:* as previously described [83,84] male BALB/c mice were injected i/p with HAF (Sigma Chemical Co.) 4 mgs in 0.1 mls of PBS i/p daily for either 6 or 13 days after which they were sacrificed. Control male BALB/c mice received PBS 0.1 mls i/p daily for 6 or 13 days and were then sacrificed. Kidneys were harvested for haematoxylin and eosin and immunoperoxidase staining. Spleen weights were measured and corrected for body weight as splenomegaly is a feature of this immune complex mediated disease[83].

INDIRECT IMMUNOPEROXIDASE STAINING

Sections of fresh frozen kidney or of other tissues were fixed in acetone, incubated with normal goat serum X 20 minutes, washed X 3 in PBS and incubated X 60 minutes with the first or test antibody. Sections were then incubated with the second antibody, either peroxidase labelled goat anti rat IgG and normal mouse serum X 60 minutes in the case of rat mAbs or peroxidase labelled goat anti mouse X 60 minutes in the case of mouse mAbs. The colour was developed by reacting with 3'3 diaminobenzidine tetrachloride and hydrogen peroxide for 10 minutes before counterstaining with haematoxylin.

To check for non-specific reactivity of the second antibody, kidney and other tissue sections from mice were stained, using the same protocol except that PBS

was used instead of the first antibody. This provided a negative control. To exclude non-specific reactivity of first antibodies of the same isotype, staining with class I mAbs of the same isotype was used as a second negative control for the tissue sections from normal mice. Staining with class I mAbs was used as a positive control for kidney and other tissue sections from IFN treated mice.

ANALYSIS OF RNA SPECIFIC FOR LY-6

Total RNA was isolated from kidney and from other tissues by the guanidinium-cesium chloride method[85]. RNA concentrations were then determined by absorbance at 260 nm. Northern blots were prepared using 15-30 μg of total RNA, electrophoresed through a 1.5% agarose 2.2M formaldehyde gel and transferred to nitrocellulose filters. The blots were then examined using 0.7 and 0.8 kb cDNA probes for Ly-6A.2 and C.2 respectively (supplied in pcEXV expression vectors by Dr R Palfree, Royal Victoria Hospital, Montreal). These probes cross-hybridize and so cannot be used to distinguish Ly-6A/E and C. A cDNA probe for either actin or glyceraldehyde phosphate dehydrogenase (GPD) was used to control for the amounts of RNA loaded when RNA from kidneys was being compared. However, when RNA from kidney was being compared to RNA from other tissues or when radiation had been administered, these controls were no longer appropriate and ethidium bromide staining of the gel was used instead to control for RNA loading. All probes were labelled with 32P. To determine relative

increases in Ly-6 specific RNA, autoradiographs of the blots were analyzed by video densitometery with integration of the area under the curve, corrected for the values obtained with the control probe.

To distinguish between Ly-6A and C, specific oligonucleotide probes (from Dr. R. Palfree) labelled with ^{32}P were used. The Ly-6A probe comprised 44 bases (5' CTAATATTGAAAGTATGGGGAGATCCTGGGTACTAAGGTCAACGT-3') concatemerised to give a 240 base pair sequence. The Ly-6C probe comprised 30 bases (5'-GGTGTGCCAATCAAGGATCCTAACATCAGG-3'). These probes correspond to the sequences where Ly-6A and C differ most.

RIBONUCLEASE PROTECTION

A ribonuclease (RNase) protection assay was done to investigate whether Ly-6C was present in kidney. Radiolabelled RNA probes were prepared from Ly-6C cDNA (supplied by Dr. R. Palfree) which was cleaved with the restriction enzyme NCO1 and transcribed using T7 and T3 RNA polymerases to give ^{32}P antisense and sense RNA probes respectively. The resulting labelled RNA probes were then purified by removal of DNA template with deoxyribonuclease, of protein with phenol and chloroform extraction and of free nucleotides with ethanol precipitation in the presence of 2M ammonium acetate. These Ly-6C sense and antisense RNA probes were hybridized for 16 hours at 44° C to RNA extracted

from kidneys of A.D.2-Ly-6b mice. The hybridizing reactants were then treated with ribonuclease A to remove the remaining free probe, thus leaving intact fragments of probe anneled to complementary sequences in the kidney RNA. These fragments were treated with proteinase K and phenol extracted to inactivate the ribonuclease, recovered by ethanol precipitation and separated by electrophoresis on an 8% denaturing polyacrylamide gel. The gel was then dried and exposed to x-ray film overnight. The relevant RNA in the samples was looked for by checking for the appropriately sized fragment of the probe on the radiograph. As controls, antisense and sense RNA probes were reacted with tRNA instead of kidney RNA and were also used on their own without ribonuclease.

CD59 IN NORMAL AND DISEASED HUMAN KIDNEY

CD59 expression in human kidneys was investigated using the YTH mAb (Cedarlane, Mississauga, Ontario). This mAb, first described by Davies et al[54], is a rat anti-human IgG. Staining was done using the same methodology described for Ly-6 staining with the YE3 mAb in mice. That is, sections of fresh frozen kidney were fixed in acetone, incubated with normal goat serum X 20 minutes, washed X 3 in PBS and incubated X 60 minutes with the YTH mAb. Sections were then incubated with the peroxidase labelled goat anti rat IgG second antibody X 60 minutes. The colour was developed by reacting with 3'3 diaminobenzidine tetrachloride and hydrogen peroxide for 10 minutes before counterstaining with haematoxylin.

To examine CD59 expression in normal kidneys, we used peroperative frozen section biopsies from donor kidneys that had just been transplanted into recipients. Obviously, such kidneys are not really normal, having undergone harvesting and periods of warm and cold ischaemia as well as possible insults during the donor's agonal illness. In an alternative attempt to examine CD59 expression in normal kidneys, we used biopsies from patients with minimal change disease which are considered to be morphologically normal except for the fusion of glomerular epithelial podocytes seen on electron microscopy. It can again be argued that these kidneys are not really normal. However, in the absence of samples of truly normal renal tissue, these two sources represented the closest approximation to normal available.

CD59 expression in diseased kidneys was looked at by examining frozen tissue sections from biopsies taken from patients with a variety of renal diseases. These included acute tubular necrosis (ATN), both in native kidneys and in grafts, acute interstitial nephritis (AIN), acute graft rejection (AR) and glomerulonephritis (GN).

TABLE 1

Oxazolone:	100 μl 25% oxazolone in acetone topically to shaven back on day 1 and 25 μl 5% oxazolone in acetone topically to shaven back on day 4. Sacrificed on day 6.
LPS:	25 mg i/p days 1 and 4. Sacrificed on day 6.
Poly I/C:	100 μg i/p day 1. Sacrificed on day 4.
P815:	20 million cells i/p on day 1. Sacrificed on day 4.
Control:	Topical acetone to shaven back days 1 and 4. PBS 0.1 mls i/p days 1 and 4. Sacrificed on day 6.

CHAPTER THREE

RESULTS

LY-6 EXPRESSION IN NORMAL KIDNEY

In order to study distribution of expression of Ly-6 in normal kidney, we performed immunohistochemistry using Ly-6 mAbs. On indirect immunoperoxidase staining of kidneys from normal A.D2-Ly-6b mice, Ly-6A.2 expression was detected with both mAbs (YE3 and 34-11-3) on the endothelium of arterioles and venules but not on that of glomeruli or capillaries (Plate 1). There was also prominent expression on the epithelium of distal tubules and collecting ducts in the renal medulla (Plate 2) and, to a lesser degree, in the renal cortex (Plate 1). Expression was most prominent in the outer zone of the medulla. In general, the staining of distal tubular epithelial cells took on a diffuse cytoplasmic pattern. Proximal tubules were mainly negative but sometimes stained faintly in a brush border pattern (Plate 1). Interstitial cells did not stain.

In normal CAF1 and BALB/c mice, similar expression of Ly-6E.1 on vascular endothelium was seen using the YE3 mAb. However, tubular staining differed from that of Ly-6A.2 in being almost entirely cortical rather than medullary (Plate 2). It also predominantly involved distal tubules and collecting ducts. Staining with the

34-11-3 mAb which only detects Ly-6A.2 was negative as expected.

Ly-6C.2 could not be detected in the kidneys of A.D2-Ly-6b mice using the 34-2-11 mAb, as staining was no different to that seen with PBS followed by the goat anti mouse second antibody alone[86].

Staining with PBS followed by peroxidase labelled goat anti rat antibody was completely negative (Plate 3). Staining with PBS followed by peroxidase labelled goat anti mouse antibody was mildly positive only in glomeruli, as has previously been described[86]. Class I staining was negative (same as control) with the H-2K, H-2D and M1 mAbs.

We then examined the expression of Ly-6 RNA in normal kidney. High steady state RNA levels for Ly-6 were detected in kidney, compared to liver, heart, spleen, brain and other organs in normal mice from both strains studied (Plate 4). Specific oligonucleotide probing showed that both Ly-6A and C RNA were present in kidney.

RNase protection assay was carried out using sense and antisense Ly-6C RNA probes in order to confirm the detection of Ly-6C in the kidney by oligonucleotide probing. Kidney tissue RNA was protected from RNase digestion by the Ly-6C cDNA antisense probe but not by the sense probe (Plate 5). This

supports the finding that Ly-6C is present in the kidney.

RADIATION AND Ly-6

In view of the intense tubular epithelial Ly-6 expression noted on mAb staining, the possibility that this represented tubular resorption as distinct from synthesis of Ly-6 was considered. If epithelial or endothelial cells are the main site of renal Ly-6 synthesis then renal Ly-6 RNA levels would be expected to be radioresistant, whereas if bone marrow derived cells are the main source, renal Ly-6 RNA levels should be radiosensitive. Ly-6 steady state RNA levels in normal kidney were unaffected by doses of radiation from 500- 2000 rads, suggesting that renal Ly-6 production is mainly by epithelial and endothelial cells (Plate 6).

Ly-6 AND IFN-γ

As regulation of Ly-6 expression by IFN-γ has been reported in lymphocytes[48], we next explored the possibility of such regulation in renal epithelium and endothelium *in vivo*. Recombinant IFN-γ and the various IFN inducing agents had similar effects on the distribution of Ly-6A.2/E.1 in kidney tissue. On immunoperoxidase staining, increased intensity of endothelial and tubular expression was seen in all murine strains tested. There was persistence of the pattern of mainly medullary tubular expression in the Ly-6A.2 strain and of

mainly cortical tubular expression in the Ly-6E.1 strains (Plate 7). The most notable effect of IFN-γ was in the renal cortex where proximal tubular epithelial staining became markedly more prominent and widespread in all strains and took on a predominantly brush border pattern of distribution within the epithelium (Plate 7). No cellular infiltrate was seen and glomerular and interstitial staining remained negative. Staining for Ly-6A.2 was again appropriately negative in the Ly-6.1 murine strains. Ly-6C.2 could not be detected in IFN induced kidneys from A.D2-Ly-6b mice using the 34-2-11 mAb.

Staining with PBS followed by the immunoperoxidase labelled antibody was negative, as in the untreated mice. Staining with the class I mAbs (MI, H-2K and H-2D) was strongly positive in the well described distribution on vascular endothelium and on the antiluminal surface of proximal tubular epithelium (Plate 8)[87], thus confirming the efficacy of the recombinant IFN-γ and of the various IFN inducing agents.

Treatment with recombinant IFN-γ markedly increased expression of steady state RNA levels for Ly-6. The time course of the increase showed that the response is early, being maximal at 6 hours, and back to baseline levels by 48 hours (Plate 9). As measured by densitometry, the increase in expression is 11 fold at 6 hours and 7 fold at 24 hours. Specific oligonucleotide probing showed that both Ly-6A and C expression are upregulated by IFN-γ.

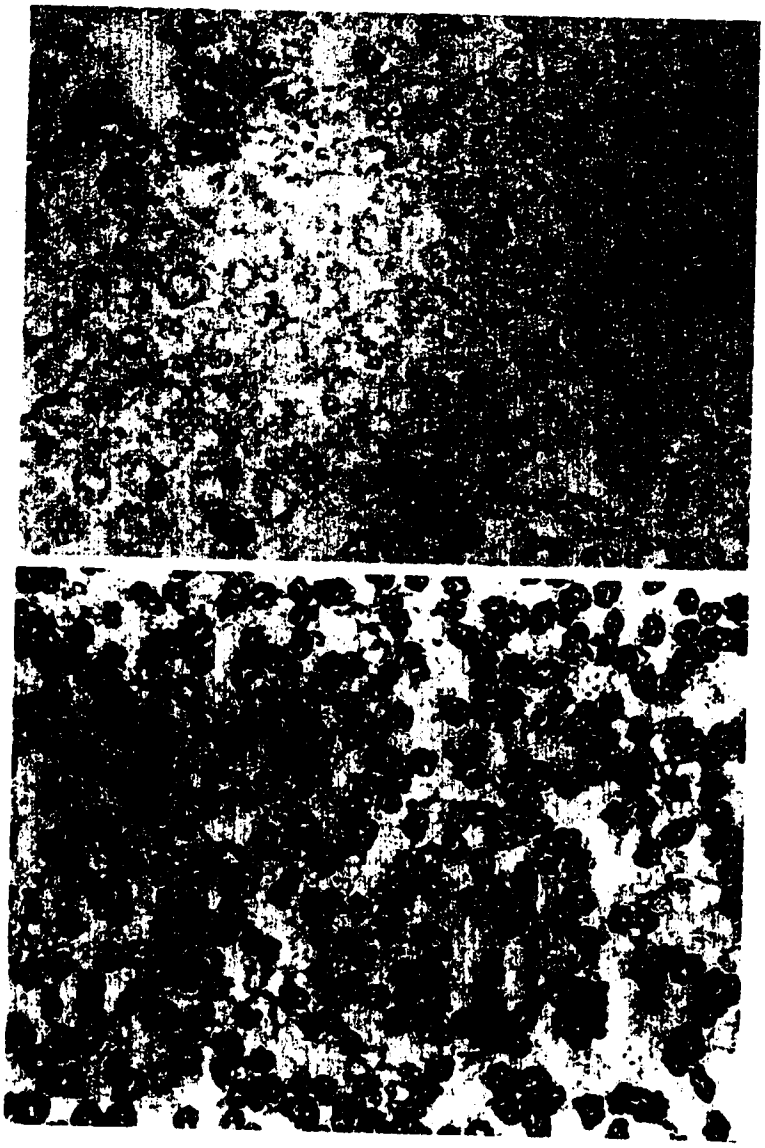
PLATE 1

X 100

X 240

Ly-6A/E EXPRESSION IN NORMAL KIDNEY CORTEX

PLATE 2



X 100

BALB/c

(Ly-6.1)

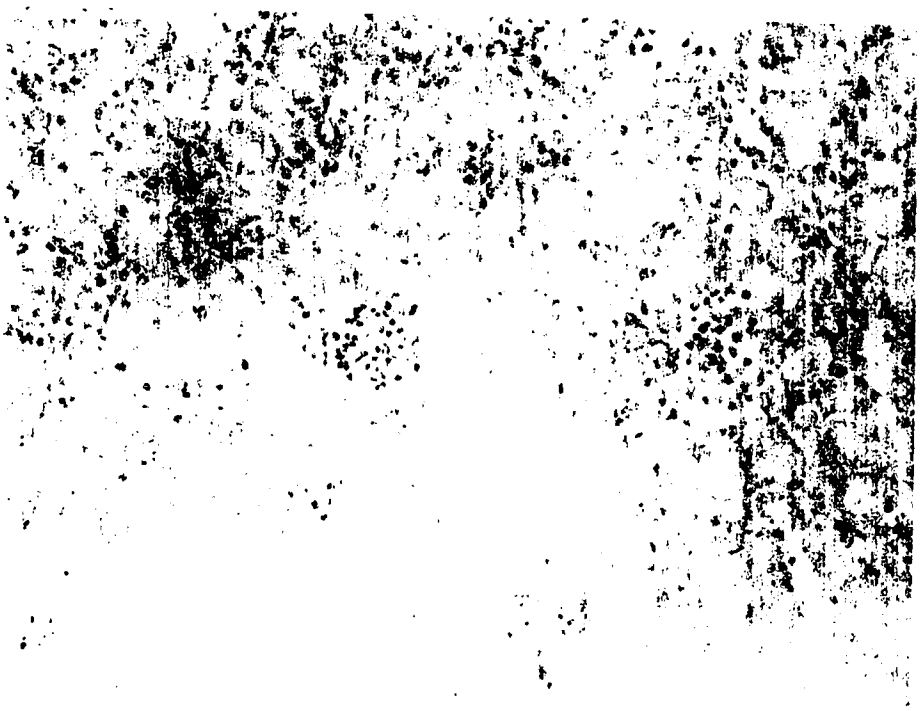
X 100

A.D2-Ly-6b

(Ly-6.2)

Ly-6A/E EXPRESSION IN NORMAL RENAL MEDULLA

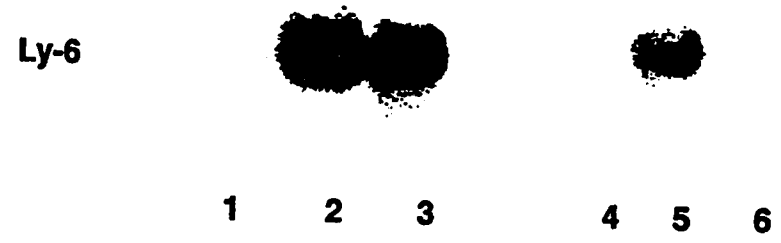
PLATE 3

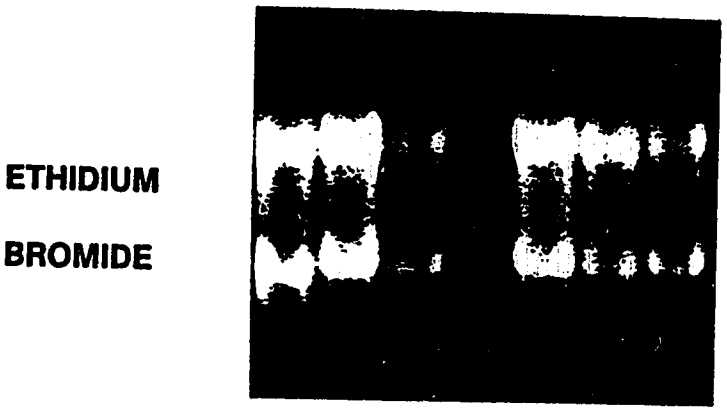


X 100

STAINING USING PEROXIDASE LABELLED ANTIBODY ONLY

PLATE 4

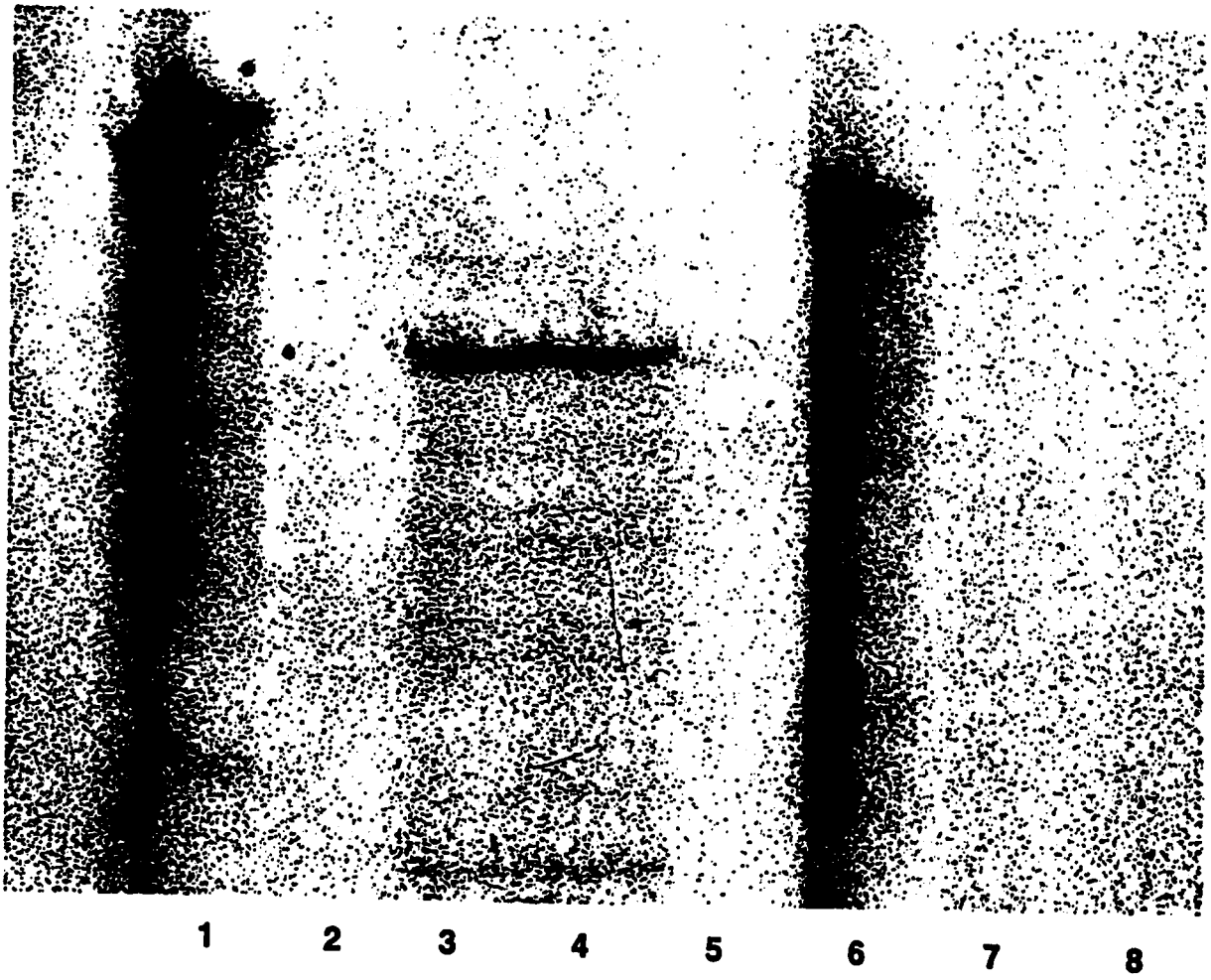




1. Spleen
2. Kidney
3. Liver
4. Salivary Gland
5. Brain
6. Heart

NORTHERN BLOT COMPARING Ly-6 RNA EXPRESSION IN KIDNEY AND OTHER ORGANS

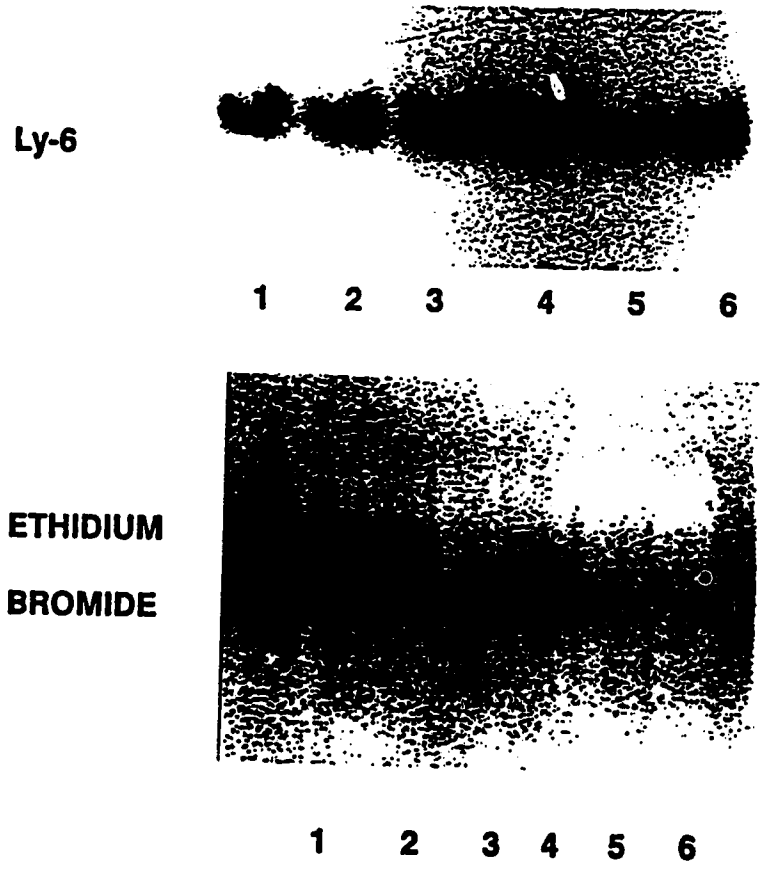
PLATE 5



1. Ly-6C antisense probe alone
2. tRNA with antisense probe
3. Kidney RNA with antisense probe
4. Kidney/LPS with antisense probe
5. Ly-6C sense probe alone
6. tRNA with sense probe
7. Kidney RNA with sense probe
8. Kidney/LPS with sense probe

RIBONUCLEASE PROTECTION ASSAY SHOWING PRESENCE OF Ly-6C RNA IN KIDNEY

PLATE 6

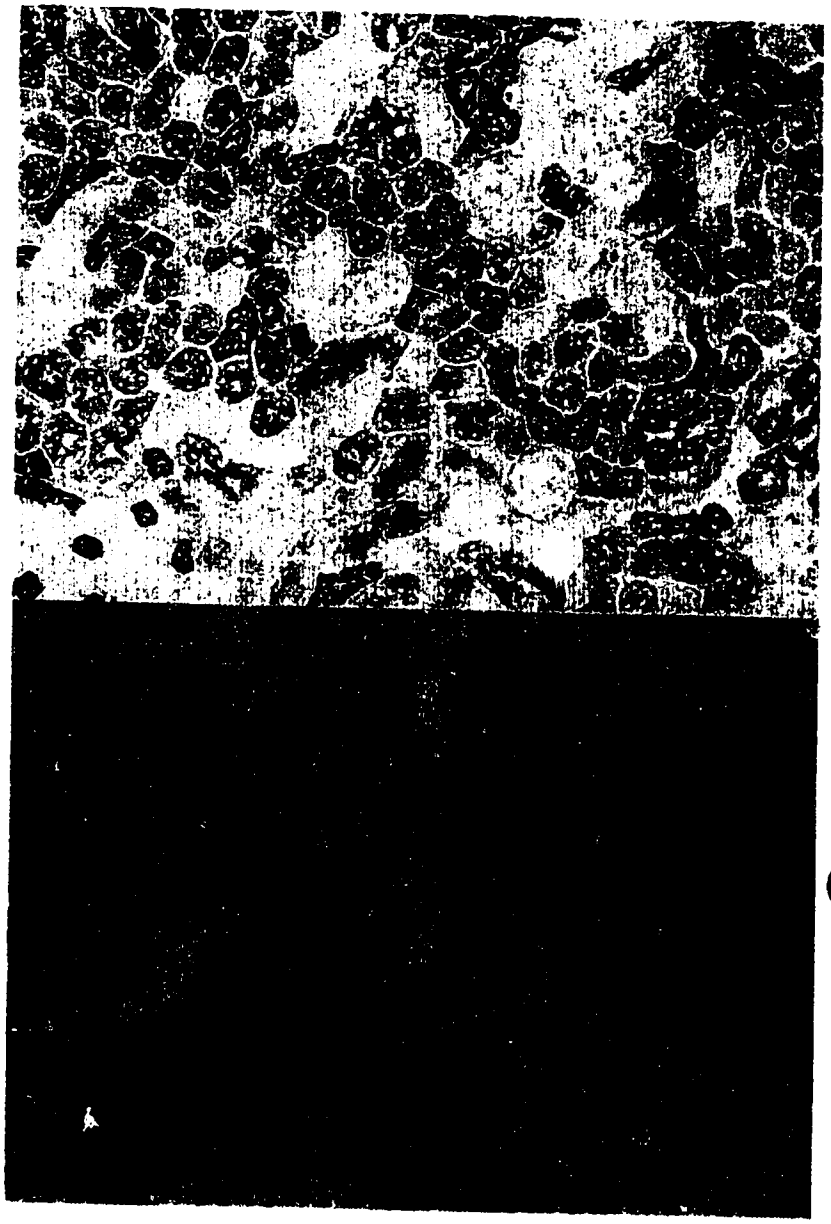


1. Sham
2. 500 Rads
3. 750 Rads
4. 1,000 Rads
5. 1,500 Rads
6. 2,000 Rads

NORTHERN BLOT SHOWING RESISTANCE

OF RENAL Ly-6 RNA TO RADIATION

PLATE 7



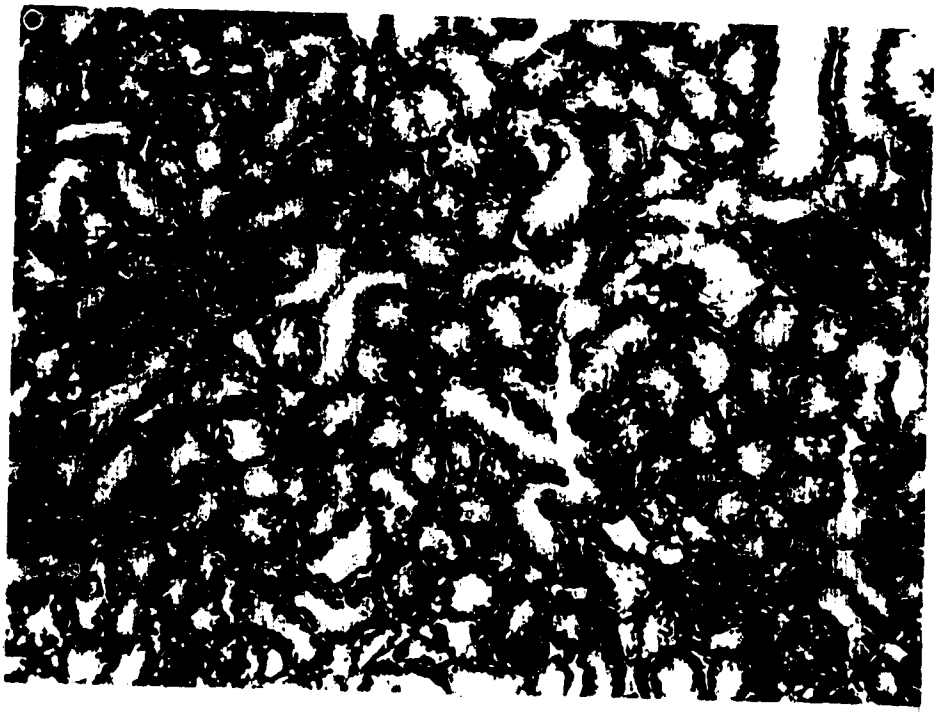
X 100

X 240

(Arrow shows luminal Ly-6)

Ly-6A/E EXPRESSION IN KIDNEY CORTEX AFTER IFN-γ INDUCTION

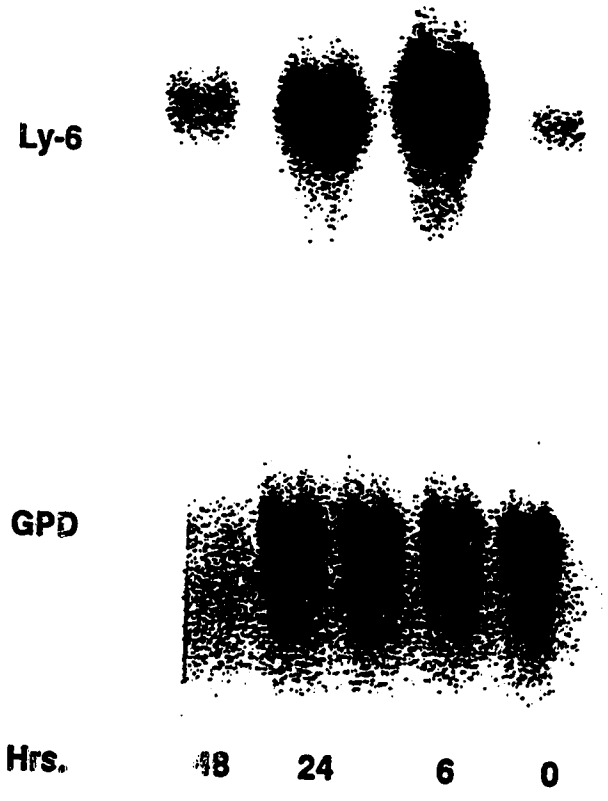
PLATE 8



X 100

CLASS I EXPRESSION IN KIDNEY AFTER IFN-γ INDUCTION

PLATE 9



NORTHERN BLOT SHOWING TIME COURSE OF Ly-6 RNA CHANGES IN KIDNEY FOLLOWING IFN-γ ADMINISTRATION

Ly-6 IN MURINE MODELS OF RENAL DISEASE

We then asked whether the same effect of IFN-γ on Ly-6 expression occurred in three *in vivo* models of murine renal disease including two where IFN-γ is known to play a role.

(1) Lupus Nephritis

There was no difference in renal Ly-6 expression, by immunoperoxidase staining, between MRL lpr/lpr mice and +/+ controls at 6 weeks of age, with both showing the same distribution of Ly-6 described for normal mice (Plate 10). Similarly, there was no difference in steady state RNA levels at 6 weeks by Northern blotting (Plate 11). However, at 17 weeks when lymphocytic infiltration is apparent in kidneys of lpr/lpr mice, immunoperoxidase staining showed a marked increase in Ly-6 expression in these mice, compared to +/+ controls. This increase was most apparent in proximal tubules where it adopted the same luminal pattern described in the IFN-γ treated mice. It was also apparent that the lymphocytic infiltrate in the kidneys of these mice expressed Ly-6. Similarly, at 17 weeks, lpr/lpr kidneys showed markedly increased Ly-6 steady state RNA levels compared to +/+ kidneys. The difference was 15-fold by densitometry (Plate 11). In view of the Ly-6 positive lymphoid infiltrate, this increase in renal Ly-6 RNA levels could not be attributed solely to increased renal epithelial and endothelial Ly-6

production.

(2) Mercuric Chloride Nephropathy

In mercuric chloride nephropathy, increased Ly-6 expression as measured by mAb staining, compared to sham treated mice, was seen in kidneys of mice who received 2 and 3.2 mgs/kg, but not of those who received 1.6 mgs/kg, 3 times weekly for one week. Again, the increased expression was most apparent in proximal tubules in a luminal distribution. In this model no lymphoid infiltrate was evident (Plate 12). Steady state RNA levels for Ly-6 were increased, relative to kidneys from sham treated animals, in kidneys from mice treated with 2.0 mgs/kg, but not in those from mice treated with 1.6 mgs/kg, mercuric chloride (Plate 13). The increase was less pronounced than that seen in the lupus nephritis model, being two-fold by densitometry. The absence of significant lymphocytic infiltration in these mice suggests that there is increased renal epithelial and endothelial Ly-6 production in this model. Kidneys from mice treated with 3.2 mgs/kg mercuric chloride actually showed decreased Ly-6 RNA levels relative to those from sham treated mice, presumably due to the ATN seen with this dose of mercuric chloride (Plate 13). It should be noted that the use of actin as a control for RNA loading is inaccurate in the presence of ATN as actin production appears to increase in this condition.

(3) Horse Apoferritin Nephritis (HAF)

Mice receiving HAF showed increased spleen to body weight ratio, compared to controls, after both one and two weeks of treatment (Table 2). On haematoxylin and eosin staining, no morphologic changes were seen at 1 week and only a moderate amount of glomerular necrosis was seen at 2 weeks.

No alteration in Ly-6 expression from normal was seen in kidneys from mice with HAF nephritis at either of the time points examined. Similarly, no alteration in MHC class I expression was seen at either time point, suggesting that IFN-γ and the proteins that it regulates may not play an important role in the pathogenesis of this condition.

OTHER EXTRALYMPHOID LY-6

As shown in Plate 4, extralymhoid Ly-6 expression was most marked in kidney but was also prominent in other tissues, especially liver, brain, heart and salivary gland.

(1) Liver: Ly-6A/E expression in liver was detected using both the YE3 and 34-11-3 mAbs. It was seen on vascular endothelium and on the epithelial lining of some of the biliary ductules but not on hepatic parenchymal cells. Its ductular

expression appeared to be on the luminal aspect of the epithelium. After treatment with IFN-γ or IFN inducers the expression of Ly-6A/E on the ductules became notably more intense and widespread but hepatic parenchymal cells remained negative (Plate 14). Ly-6C expression was not detected in liver using the mAb. When cDNA probing of Northern blots was used to investigate the time course and intensity of hepatic Ly-6 upregulation in response to IFN-γ, results similar to those for renal Ly-6 were apparent. Thus upregulation was rapid, being most intense at 6 hours, less intense at 24 hours and back almost to baseline at 48 hours (Plate 15). The maximal increase at 6 hours was X-fold by densitometry.

(2) Pancreas: Ly-6A/E expression in pancreas was on vascular endothelium and on the luminal aspect of the epithelium of pancreatic ducts but not on pancreatic acini or on islets of Langerhans. Some expression was also apparent in the interstitium but the cells responsible were not identifiable. After treatment with IFN inducers, Ly-6A/E upregulation was apparent on pancreatic duct epithelium where it became both more widespread and more intense (Plate 16a).

(3) Heart: Ly-6A/E expression in heart was only seen on vascular endothelial tissue. It was not apparent on myocytes, in the interstitium or on endocardium (Plate 16b). Endothelial expression appeared to be more intense after treatment with IFN inducers.

(4) Salivary Gland: Ly-6A/E expression in salivary gland was detected on vascular endothelium and on some salivary gland acini and ducts. Again, ductal epithelial expression was predominantly on the luminal aspect of the epithelium (Plate 17). After treatment with IFN inducers expression was increased, particularly in the acini and on the ductular epithelium.

CD59 EXPRESSION IN 'NORMAL' HUMAN KIDNEY

Renal tissue was examined from perioperative biopsies from 3 transplant donor kidneys and from 2 patients with minimal change disease. In all cases staining with PBS instead of the first antibody, followed by the peroxidase labelled goat anti-rat second antibody was negative. In all cases the YTH mAb showed positive staining indicating the presence of CD59 on vascular endothelium and on tubular epithelium, particularly in the distal nephron, just as was described for Ly-6 in mouse kidney. This epithelial CD59 was predominantly seen on the luminal aspect of tubular epithelium. In addition, CD59 was apparent in glomeruli, both on glomerular capillary endothelium and in the glomerular mesangium. No staining was seen in the renal interstitium (Plate 18).

CD59 EXPRESSION IN DISEASED HUMAN KIDNEY

No differences were apparent in CD59 expression when 3 biopsies from

patients with ATN and two biopsies from patients with GN due to immunoglobulin A nephropathy were compared with the 'normal' kidney biopsies described above (Plate 19). However, kidney biopsies from the 2 patients with AIN and from the 3 patients with AR showed more intense and widespread tubular expression of CD59 than was seen in the 'normal' kidneys (Plate 20). Proximal tubular CD59, in particular, became more prominent. In addition CD59 staining of the lymphoid infiltrate in both these conditions was seen.

CHAPTER FOUR

DISCUSSION

We have shown, using both mAbs and cDNA probes, that Ly-6 A.2/E.1 is strongly epressed in kidney, on both tubular epithelium and vascular endothelium, is radiation resistant and can be regulated by both IFNs-α/β and γ. The endothelial expression is on small arteries, arterioles and veins but not on glomeruli or capillaries. It does not differ from endothelial expression in other organs. In contrast, the intense expression in renal epithelium is unique. It is allele specific in that its pattern of distribution between cortex and medulla varies markedly between murine strains expressing the two distinct Ly-6 alleles, A.2 and E.1. This seems surprising but a similar variation in expression on peripheral lymphocytes has been reported for the same two alleles[1,2]. Renal expression is also locus specific in that it appears to be accounted for predominantly by Ly-6A.2 and E.1 rather than by Ly-6C. Again, similar variations in expression between Ly-6A/E and C have been reported in lymphoid tissue[1,2]. The mechanisms underlying these differences in gene expression are unknown.

The apparent discrepancy between the presence in kidney of Ly-6C RNA on oligonucleotide probing and RNase protection assay and the absence of Ly-6C product on immunoperoxidase staining may be due to the levels of the latter being

undetectably low or to alteration in kidney of the particular epitope recognised by the 34-2-11 mAb.

Ly-6 AND INTERFERON

The induction of Ly-6 in kidney by IFN-γ occurs rapidly and is not related to any interstitial infiltrate. Rather, it appears to be due to increased intensity of both epithelial and endothelial expression. The presence of the same pattern of upregulation in two models of murine renal disease in which IFN-γ is known to play a role indicates that IFN-γ regulation of Ly-6 is likely to have *in vivo* relevance. IFN regulation of Ly-6 has previously been described in lymphocytes[48,49]. An IFN-responsive sequence, analogous to that found in association with MHC class I and II genes, has been identified upstream to one of the successfully sequenced Ly-6 genes[24]. Other IFN inducible structures in kidney include MHC class I and II, β-2-microglobulin and the adhesion molecule, ICAM-1[85,88].

The predominantly distal expression in the nephron contrasts with that of other immunologically important antigen systems expressed on tubules, such as MHC class I and II and ICAM-1, which have a predominantly proximal distribution[85,88].

GPI ANCHORED PROTEINS AND APICAL TARGETING

When proximal tubular Ly-6 expression does become prominent, after IFN induction, it follows a luminal or brush border pattern. This contrasts with the IFN induced pattern for MHC class I and II, which is antiluminal i.e. basolateral[85], but is similar to the pattern found with ICAM-1[89].

Interestingly, it has recently been demonstrated in a renal epithelial cell line that membrane proteins with a GPI anchor are always expressed on the apical, and never on the basolateral, surface of the cell, suggesting that attachment of the GPI anchor to the precursor protein in the endoplasmic reticulum leads to specific apical targeting of the resulting product[90-92]. Ly-6 antigens all have a GPI anchor and so it is not surprising that we have found that Ly-6A.2 and E.1 localize to the luminal surface, after IFN induction. It appears that this apical targeting of GPI anchored proteins is a feature of other polarised epithelia also and this is supported by our detection of Ly-6 on the luminal surface of salivary, biliary and pancreatic and ductal epithelium.

The mechanism of this apical targeting remains unclear but recent evidence suggests that sorting of GPI anchored proteins to glycosphingolipid-enriched membrane subdomains occurs in the Golgi apparatus. These sphingolipids are also known to be preferentially apically targeted and they may act as carriers to

bring the GPI anchored protein in the same direction[93]. The purpose of this apical targeting is also unclear but presumably it relates to the function of these proteins and of their anchors. Thus, some such as alkaline phosphatase and 5'nucleotidase, have a recognised enzymatic function which may be required on only one aspect of a polarised epithelium. However, why complement regulatory proteins or T cell activation molecules should have such selective expression remains a mystery.

GPI anchors may confer other important properties on cell surface proteins such as enhanced lateral mobility in the cell membrane, facilitation of rapid shedding by appropriate phospholipase enzymes and the release of potential second messengers, such as diacylglycerol, when the anchor is cleaved off[18-20].

IS CD59 THE HUMAN EQUIVALENT OF Ly-6?

Given the high basal levels of expression of Ly-6, it is reasonable to speculate that a human homologue will eventually be found. Analogous multigene systems have been described in sheep and rat[28,29] and, indeed, in the latter case the relevant cDNA sequences were isolated from kidney tissue. Recently, two newly characterised human GPI anchored cell membrane proteins, CD59 and uPAR have been shown to have considerable DNA sequence homology with Ly-6 and have been proposed as members of a new Ly-6 'superfamily'[30,31].

CD59 has as its function homologous restriction of the cytolytic action of the membrane attack complex of complement[64]. It is of particular interest in relation to Ly-6 because, contrary to an initial report[58], it is extensively expressed in kidney[70-72] . The distribution of renal CD59 expression appears to differ with the antibody used and between normal and diseased kidneys and has been variously reported to involve vascular endothelium, glomerular and peritubular capillaries and both distal tubules and collecting ducts[70-72]. The pattern that we have detected for CD59 in kidney is strikingly similar to what we have described for Ly-6A/E. The main difference is that CD59 has glomerular endothelial and mesangial expression, unlike Ly6 A/E. It is worthwhile noting at this point that DAF, which is another GPI-anchored homologous restriction factor for complement, has also been shown to be expressed on renal tubular epithelium and in the juxtaglomerular apparatus[72,94]. Moreover, CD59 and DAF are both expressed on T cells and mAbs against them are, like those against Ly-6, capable of mediating T cell activation[47,68].

In view of the similarities between Ly-6 and CD59 in terms of structure, signal transduction capabilities and renal expression, it seems reasonable to speculate that CD59 is the human homologue of Ly-6, and that the latter may also be involved in complement restriction. However, CD59 differs from Ly-6 in being encoded by a single gene rather than by a multigene family, in being polymorphic and in not being IFN regulated[58,70]. Also, amino acid homology between these two proteins is only 26% and their tissue distribution differs in that Ly-6, unlike CD59,

is not detected on erythrocytes, a surprising finding for a molecule with a possible complement restricting function. Thus it may be safer to conclude that Ly-6 and CD59 are relatives rather than exact equivalents[70].

FUNCTION OF Ly-6

The function of Ly-6 is unclear. It is possible that, like CD59 and DAF, it is involved in protecting lymphoid cells and renal and other epithelial and endothelial cells from complement mediated attack. If this is so, its presence on the luminal surface of tubular epithelium is somewhat surprising and suggests that it inhibits the action of complement in urine. At present however, there is no evidence apart from its similarity to CD59 that Ly-6 has a complement regulatory function.

The hypothesized role of Ly-6 in T cell activation might be consistent with a complement regulating function as both might be considered appropriate responses to a pathogen. However, the strong evidence that Ly-6 has a role in T cell activation must be be qualified by the observation that mAbs to many other GPI linked proteins, including 5'nucleotidase, Thy-1 and Qa-2, as well as CD59 and DAF can also activate T cells, raising the issue of whether this is artifactual rather than physiological[46,47,95,96]. Recent evidence indicates that the ability of GPI anchored proteins to activate T cells may relate to their association, perhaps via their anchors, with intracellular protein tyrosine kinases similar to those involved in

signal transduction from other receptors[97]. Both Ly-6 and CD59 are now known to have such an association with tyrosine kinases and so this strengthens the case that they have some role in cell activation, at least in the broad sense of the term.

A LIGAND FOR Ly-6?

Clues to the identity of the natural ligand for Ly-6 may be derived from looking at the structures of known ligands for other members of the Ly-6 superfamily. The natural ligands for CD59 and urokinase plasminogen activator receptor (uPAR) are the C5-8 complex of the complement system and uPAR respectively. Each of these has a receptor binding region with homology to the growth factor module initially described in the epidermal growth factor molecule, but also found in a variety of other molecules including coagulation factors IX and X, transforming growth factor alpha, low density lipoprotein receptor and selectin adhesion molecules[31,59,97]. Palfree has recently speculated that the natural ligand for Ly-6 may similarly contain an epidermal growth factor module and that a review of molecules known to include this module might be informative[31]. He has also noted that one such molecule is THGP, also known as uromodulin, which is present in large amounts in normal urine[31,98]. This is of interest in view of our findings that Ly-6 expression is prominent on renal tubules, especially distally where THGP is most abundant, and that IFN induced Ly-6 is mainly on the apical surface of tubular epithelium where it is exposed to potential luminal ligands, such

as THGP, rather than to blood-borne ones. THGP has no clearly proven physiological function although it is known to have immunosuppressive properties[99]. A potential interaction between it and renal tubular Ly-6 merits investigation.

Ly-6, A TRANSPLANT ANTIGEN?

The strong antigenicity of Ly-6, its susceptibility to IFN regulation, its polymorphism and its presence on endothelium, as well as the allele specific variation in its expression, make it a unique candidate to be an important antigen system in transplantation. There has long been speculation about a non MHC endothelial antigen system which might evoke destructive antibody responses in allograft rejection but no such system has been convincingly characterised[100]. This potential role of Ly-6 as a transplantation antigen would, of course, be greatly strengthened if a human equivalent were discovered that, unlike CD59, was both polymorphic and IFN regulated. For the present however, there is no direct evidence that Ly-6 has such a role.

CD59 IN KIDNEY

The extensive distribution of CD59 expression in kidney is quite surprising, in the light of the evidence that this protein's function is homologous restriction of

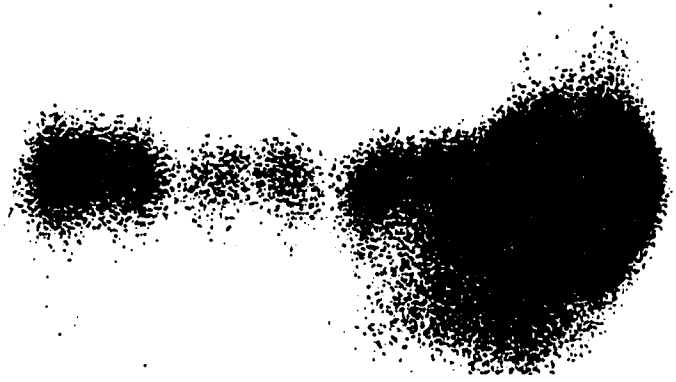
the membrane attack complex of complement. In particular, the expression on the luminal aspect of the tubular epithelium would seem to suggest that it is protecting against urinary complement, a somewhat surprising notion! This is supported by the fact that another important complement regulator, DAF, has a similar renal tubular luminal distribution[72]. There are of course alternative possibilities. One is that this expression of CD59 and DAF has no actual function and is merely a consequence of them both possessing a GPI anchor, which they have because of an actual function elsewhere. However, such a hypothesis seems unlikely given the intensity of the renal expression and, in any case, it would conflict with the usual tendency of nature not to be wasteful and express proteins where they have no function! A more likely alternative is that proteins such as DAF and CD59 have other functions, in addition to complement regulation, and that their renal tubular luminal expression is required for the mediation of one of these functions. As already mentioned, CD59 has been described by Groux et al as having functions as an adhesion molecule and as an accessory molecule in T cell activation so that the notion of multiple functions for these proteins is not unreasonable[62].

If CD59's function in kidney is complement regulation, then its alterations in the presence of renal inflammatory disease may be very informative. Our preliminary results on CD59 expression in renal disease are based on small numbers of patients and a limited range of renal pathology. However, they do suggest that an increase in both intensity and distribution of tubular epithelial

expression occurs in the presence of interstitial inflammatory disease as seen in both AIN and ATR. No alteration in CD59 was seen in ATN, GN or in minimal change disease. How upregulation of CD59 is brought about in interstitial inflammatory disease is open to speculation but initial evidence suggests that, in contrast to that of Ly-6, it is not IFN mediated[69].

PLATE 11

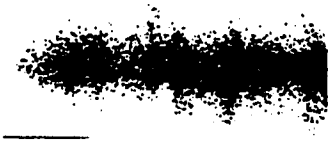
Ly-6



1 2 3 4

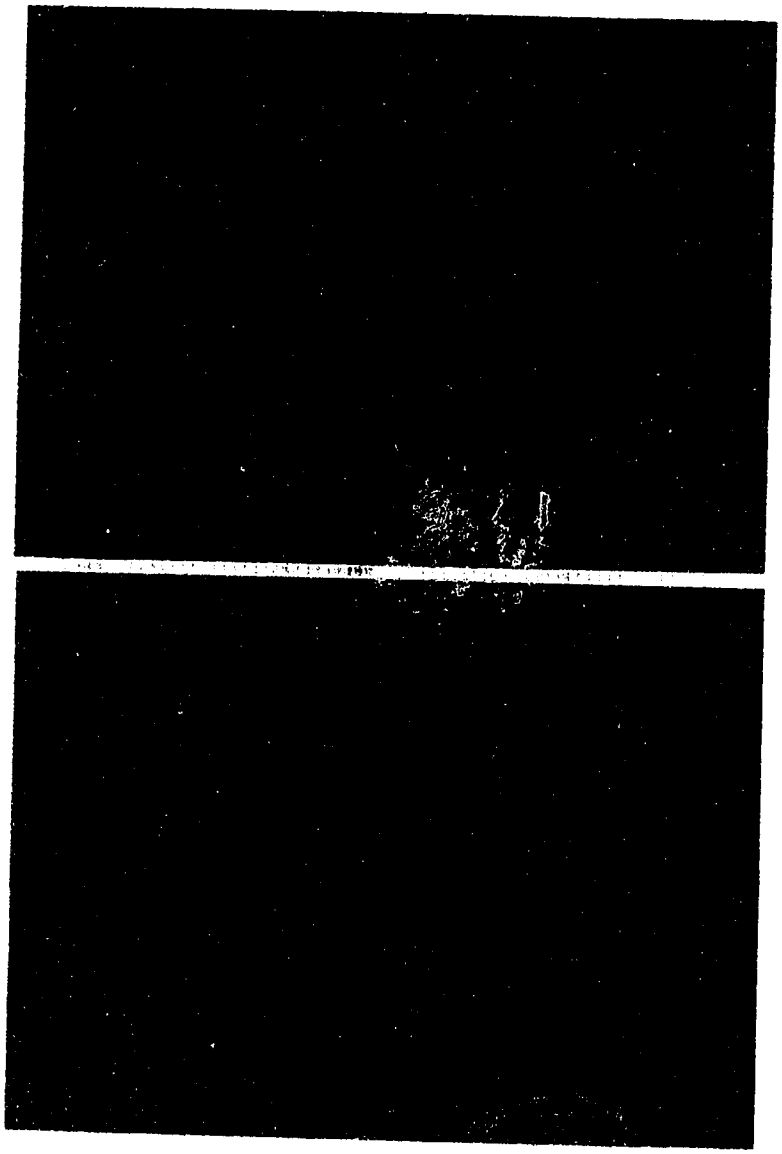
1. +/+ 6 weeks
2. lpr/lpr 6 weeks
3. +/+ 17 weeks
4. lpr/lpr 17 weeks

ACTIN



NORTHERN BLOT SHOWING Ly-6 RNA LEVELS IN MRL +/+ lpr/lpr KIDNEYS AT 6 AND 17 WEEKS

PLATE 12



(A) X 100

(B) X 100

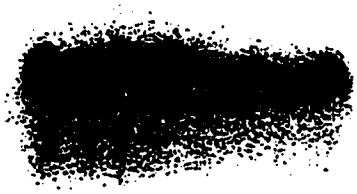
Ly-6A/E EXPRESSION IN KIDNEYS OF (A) SHAM AND (B) MERCURIC CHLORIDE (2 mg/kg) TREATED MICE AFTER ONE WEEK

PLATE 13

Ly-6

1 2 3 4

ACTIN



1. Sham
2. $HgCl_2$ 1.6 mg/kg
3. $HgCl_2$ 2.0 mg/kg
4. $HgCl_2$ 3.2 mg/kg

NORTHERN BLOT SHOWING Ly-6 RNA EXPRESSION IN MERCURIC CHLORIDE AND SHAM TREATED MICE

TABLE 2

HORSE APOFERRITIN-INDUCED IMMUNE COMPLEX NEPHRITIS

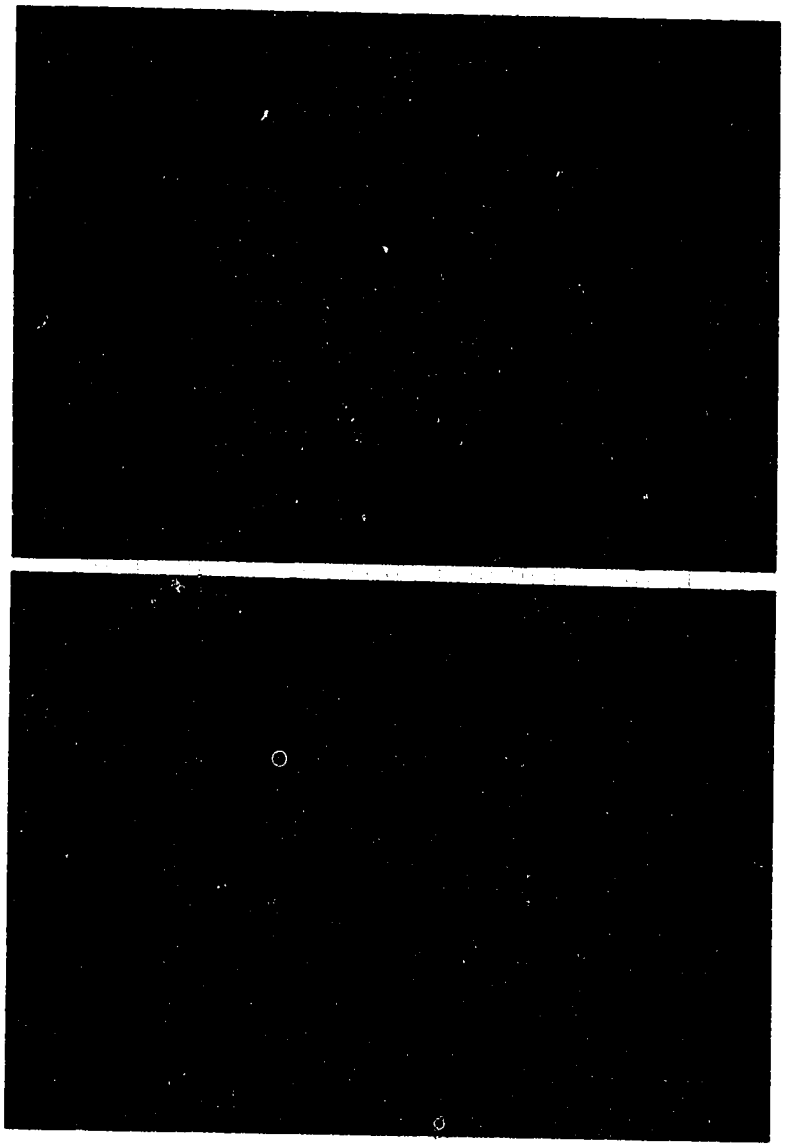
SPLEEN/BODY WEIGHT RATIOS

Group	Treatment	No. of Mice	Ratio
1	SHAM x 6 days	7	4.7 ± 0.2
2	HAF x 6 days	7	5.3 ± 0.2*
3	SHAM x 13 says	8	4.8 ± 0.3
4	HAF x 6 days	7	6.8 ± 0.2**

* $P < 0.05$ COMPARED TO SHAM

** $P < 0.01$ COMPARED TO SHAM

PLATE 14



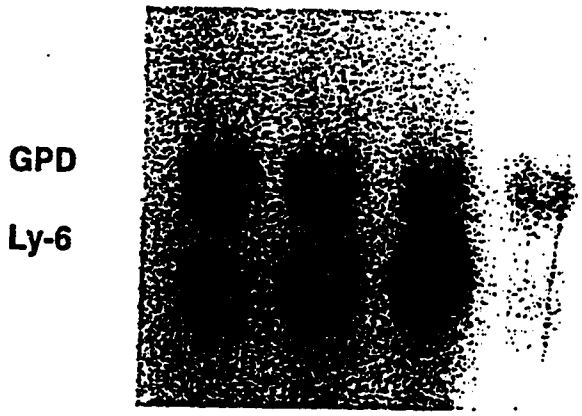
(A) X 100

(B) X 240

Ly-6A EXPRESSION IN (A) NORMAL LIVER AND

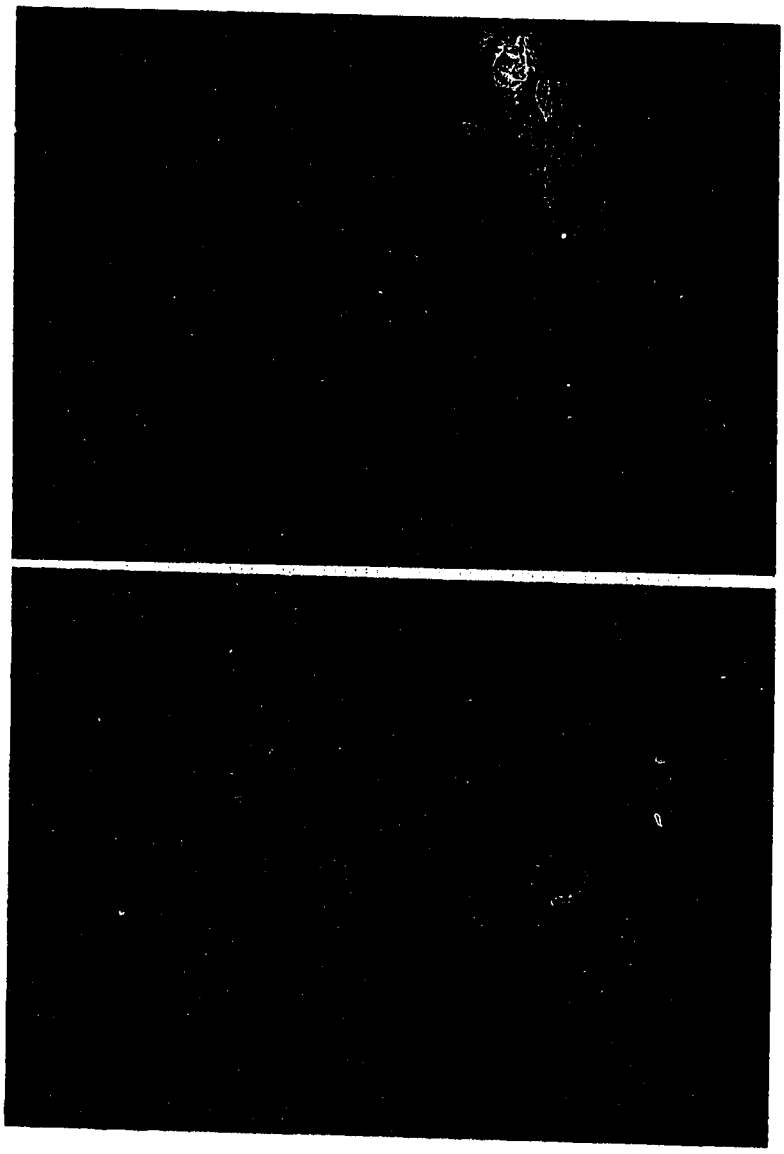
(B) LIVER AFTER IFN-*γ* INDUCTION

PLATE 15



NORTHERN BLOT SHOWING TIME COURSE OF Ly-6 RNA CHANGES IN LIVER AFTER IFN-*γ* ADMINISTRATION

PLATE 16



(A) X 100

(B) X 240

Ly-6A EXPRESSION IN (A) HEART AND (B) PANCREAS

PLATE 17

X 240

Ly-6A EXPRESSION IN SALIVARY GLAND

PLATE 18

X 160

Transplant

Donor

X 400

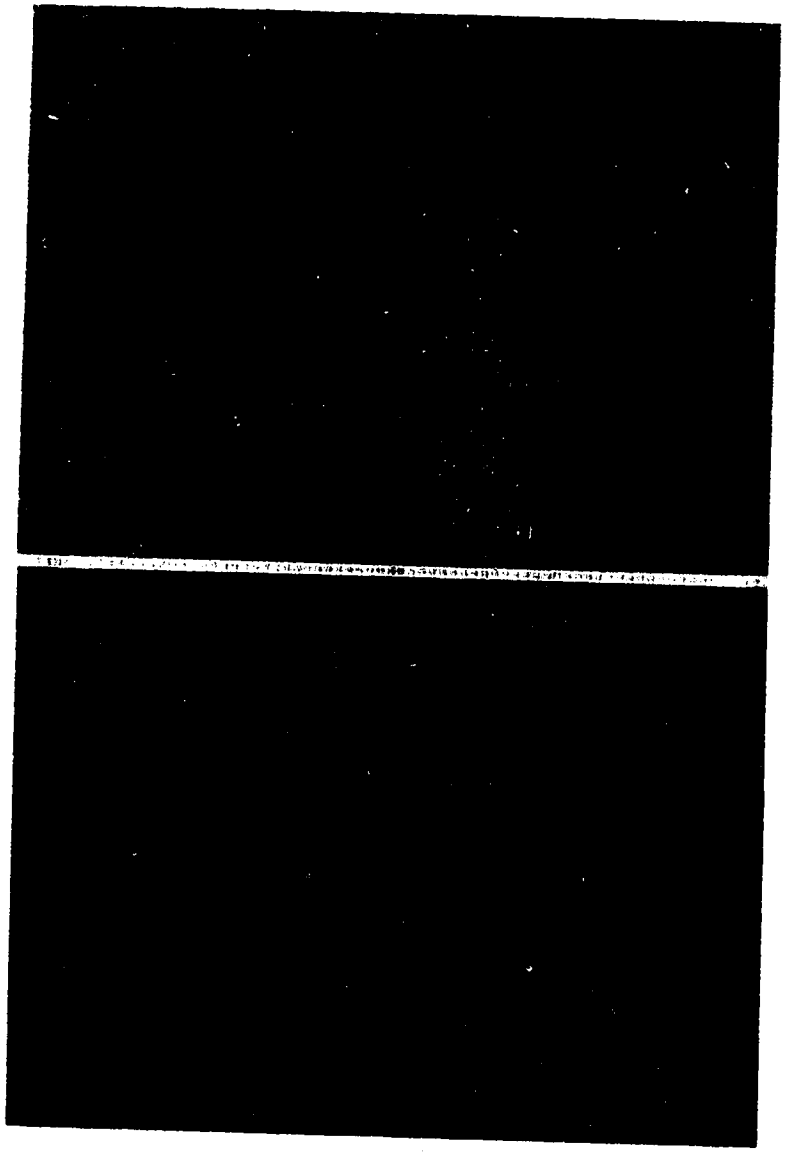
Minimal

Change

Disease

CD59 EXPRESSION IN 'NORMAL' HUMAN KIDNEY

PLATE 19



X 100

X 160

CD59 EXPRESSION IN GN (IgA NEPHROPATHY)

PLATE 20

X 160

X 160

CD59 EXPRESSION IN AR AND AIN

CHAPTER FIVE

CONCLUSION

A number of conclusions can be drawn from this body of work. Our essential finding is that Ly-6A/E is abundant in the kidney, being expressed on both vascular endothelium and tubular epithelium. The epithelial expression is of particular interest because it is predominantly in the distal tubules and collecting ducts rather than in the proximal tubules and because it shows allele specific variation in its distribution of expression between renal cortex and medulla. Renal Ly-6C expression was not detected using immunoperoxidase staining but was apparent by oligonucleotide probing and by RNase protection assay.

We have shown that Ly-6 can be upregulated by IFN-γ and that this upregulation is associated with a particular pattern of expression on the luminal or apical aspect of the proximal tubular epithelium. We have also shown that the same pattern of upregulation is seen in both murine lupus and mercuric chloride nephropathy, two conditions known to be associated with increased IFN-γ activity. In contrast, no evidence of alteration in Ly-6 expression was found in HAF immune complex nephritis, perhaps because IFN-γ is less involved in the pathogenesis of this condition.

Finally, we have shown that CD59 in human kidney has some characteristics in common with Ly-6 in murine kidney. In particular, CD59 has a similar pattern of expression including the same tendency to be on the apical aspect of tubular epithelium. We have shown preliminary evidence that CD59 may also be upregulated in certain renal diseases but not in others.

These findings are mainly of a descriptive nature and do not allow us to reach conclusions about the functions in the kidney of either Ly-6 or CD59. However, descriptive work can give clues to underlying functions and with this in mind a few speculations and suggestions for further research seem justified.

The extensive renal and the other extra-lymphoid expression of Ly-6 undermine somewhat the prevalent notion that Ly-6 is an accessory lymphocyte activation molecule. It is difficult to explain why a molecule with this function would have such a pattern of expression and it seems reasonable to suggest that Ly-6 has other functions and that the association with lymphocyte activation may represent somewhat of a 'red herring'. Support for this latter idea comes from the finding that every GPI anchored molecule tested so far, including such unlikely candidates as 5'nucleotidase and DAF, can also mediate lymphocyte activation[45,46]. These comments are also relevant to CD59 which has the same ability to mediate activation but which also has a much more convincing function in regulation of complement[54,63].

Another question that needs to be answered is why complement regulatory molecules such as CD59 and DAF have their expression on the luminal rather than the basolateral surface of renal tubular and of ductal epithelium. It might have been expected that these molecules would be more likely to be found on the aspect of the epithelium that faces the blood rather than on that which is in contact with urine and other body secretions. Is this just an accident of nature? Is it a price that had to be paid for having a GPI anchor which was required for other reasons such as increased lateral mobility within the cell membrane? Alternatively, do these molecules or does the complement system they apparently regulate have unrecognised functions at these sites where they are so strongly expressed?

Obviously the identification of the natural ligand or ligands for Ly-6 will continue to be a focus for investigators in this field and will give insights into the function of this family of proteins. At the 1992 annual meeting of the American Association of Immunologists, two groups presented preliminary evidence that CD2 and immunoglobulin M-α might be Ly-6 ligands but at present the evidence for either of these hypotheses is not conlcusive and the search for ligands will continue[101,102].

The issue of whether CD59 is the human Ly-6 or whether there is a more closely related human molecule will also continue to attract attention and it is likely that the recently christened Ly-6 'superfamily' will continue to expand. The

discovery of a human molecule in this superfamily that is IFN-regulated, polymorphic and part of a multigene family would certainly provide a more convincing candidate for the human Ly-6 equivalent.

Other worthwhile projects for the future would include a detailed description of Ly-6 distribution in other tissues such as brain, bowel and lung, an investigation of alterations in Ly-6 in non-renal as well as renal disease models and an attempt to isolate Ly-6 from the urine and other body secretions in the light of these molecules' expression on epithelial surfaces. With regard to CD59, further investigation of its alterations in human renal disease seem indicated with special attention to those conditions in which the complement MAC is thought to be important. These would include membranous nephropathy[103] and post-streptococcal GN[104]. Also, the issue of whether CD59 is regulated by IFN or other cytokines should not be neglected just because of initially negative findings[696].

In conclusion, this body of work, like many others, raises more questions than it answers. This author hopes that its most important contribution will be to draw attention to aspects of Ly-6, CD59 and related molecules that have not received much attention in the past and so alter the way that investigators view these fascinating proteins, until eventually functional explanations are provided for the descriptive data detailed here.

BIBLIOGRAPHY

1. Shevach EM, Korty PE: Ly-6: a multigene family in search of a function. *Immunol Today 10:195-200, 1989*

2. Rock KL, Reiser H, Bamezai A, McGrew J, Benacerraf B: The Ly-6 locus: a multigene family encoding phosphatidylinositol-anchored membrane proteins concerned with T cell activation. *Immunol Rev 111:195-224, 1989*

3. McKenzie IFC, Cherry M, Snell GD: Ly-6.2: a new lymphocyte specificity of peripheral T cells. *Immunogenetics 5:25-32, 1977*

4. Sachs JA, Huber B, Pena-Martinez J, Festenstein H: Genetic studies and effect on skin allograft survival of DBA/2, DAG, Ly, and M-locus antigens. *Transplant Proc 5:1385-1387, 1973*

5. Feeney AJ, Hammerling U: Ala-1: a murine alloantigen of activated lymphocytes. *Immunogenetics 3:369-379, 1976*

6. Feeney AJ: Expression of Ly-6 on activated T and B cells: possible identity with Ala-1. *Immunogenetics 7:537-543, 1978*

7. Horton MA, Sachs JA: Identity of murine lymphocyte alloantigens DAG, Ala-1, Ly-8 and Ly-6? *Immunogenetics 9:273-280, 1979*

8. Potter TA, McKenzie IFC, Morgan GM, Cherry M: Murine lymphocyte alloantigens. I. The Ly-6 locus. *J Immunol 125:541-545, 1980*

9. Takei F, Galfre G, Alderson T, Lenoux ES, Milstein C: H9/25 monoclonal antibody recognizes a new allospecificity of mouse lymphocyte

subpopulations: strain and tissue distribution. *Eur J Immunol 10:241-246, 1980*

10. Kimura S, Tada N, Nakayama E, Hammerling U: Studies of the mouse Ly-6 alloantigen system. I. Serological characterisation of mouse Ly-6 alloantigen by monoclonal antibodies. *Immunogenetics 11:373-381, 1980*

11. Eckhardt LA, Herzenberg LA: Monoclonal antibodies to ThB detect close linkage of Ly-6 and a gene regulating ThB expression. *Immunogenetics 11:275-291, 1980*

12. Kimura S, Tada N, Liu-Lam Y, Hammerling U: Studies of the mouse Ly-6 alloantigen system. II. Complexities of the Ly-6 region. *Immunogenetics 20:47-56, 1984.*

13. Palfree RGE, Dumont FJ, Hammerling U: Ly-6A.2 and Ly-6E.1 molecules are antithetical and identical to MALA-1. *Immunogenetics 23:197-207, 1986*

14. Palfree R: Ly-6 nomenclature. *Immunol Today 12:45-46, 1991*

15. Takei F: Biochemical characterisation of H9/25, an allospecificity encoded by the Ly-6 region. *Immunogenetics 16:201-208, 1982*

16. Matossian-Rogers A, Rogers P, Ledbetter JA, Herzenberg LA: Molecular weight determination of two genetically linked cell surface murine antigens: Th-B and Ly-6. *Immunogenetics 15:591-599, 1982*

17. Reisser H, Oettgen H, Yeh ETH, Terhorst C, Low MG, Benacerraf B, Rock KL: Structural characterisation of the TAP molecule: a phosphatidylinositol-linked glycoprotein distinct from the T cell receptor/T3 complex and Thy-1.

Cell 47:365-370, 1986

18. Ferguson MAJ, Williams AF: Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. *Ann Rev Biochem 57:285-320, 1988*

19. Low MG, Saltiel AR: Structural and functional roles of glycosyl-phosphatidylinositol in membranes. *Science 239:268-275, 1988*

20. Low MG: The glycosyl-phosphatidylinositol anchor of membrane proteins. *Biochemica et Biophysica Acta 988:427-454, 1989*

21. LeClair KP, Palfree RGE, Flood PM, Hammerling U, Bothwell A: Isolation of a murine Ly-6 cDNA reveals a new multigene family. *EMBO J 5:3227-3234, 1986*

22. Reiser H, Coligan J, Palmer E, Benacerraf B, Rock KL: Cloning and expression of a cDNA for the T cell activating protein TAP. *Proc Natl Acad Sci (USA) 85:2255-2259, 1988*

23. Palfree RGE, Sirlin S, Dumont FJ, Hammerling U: N-terminal and cDNA characterisation of murine lymphocyte antigen Ly-6 C.2. *J Immunol 140:305-310, 1988*

24. Bothwell A, Pace PE, LeClair KP: Isolation and expression of an IFN-responsive Ly-6 C chromosomal gene. *J Immunol 140:2815-2820, 1988*

25. LeClair KP, Rabin M, Nesbitt MN, Pravtcheva D, Ruddle FH, Palfree RGE, Bothwell A: Murine Ly-6 multigene family is located on chromosome 15. *Proc. Natl. Acad. Sci. (USA) 84:1638-1642, 1987*

26. McGrew JT, Rock KL: Isolation, expression, and sequence of the TAP/Ly-6 A.2 chromosomal gene. *J Immunol 146:3633-3688, 1991*

27. Williams AF, Tse AGD, Gagnon J: Squid glycoproteins with structural similarities to Thy-1 and Ly-6 antigens. *Immunogenetics 27:265-272, 1988*

28. Hein WR, Beya MF: Evidence that the B5 molecule on sheep lymphocytes is a homologue of mouse Ly-6 and human CD59. *Immunology 68:580-582, 1989*

29. Friedman S, Palfree RGE, Sirlin S, Hammerling U: Analysis of three distinct Ly-6A related cDNA sequences isolated from rat kidney. *Immunogenetics 31:104-111, 1990*

30. Stefanova I, Hilgert I, Kristofova H, Brown R, Low MG, Horejsi V: Characterisation of a broadly expressed human leucocyte surface antigen MEM-43 anchored in the membranethrough phosphatidylinositol. *Mol Immunol 20:153-161, 1989*

31. Palfree RGE: The urokinase-type plasminogen activator receptor is a member of the Ly-6 superfamily. *Immunol Today 12:170-171, 1991*

32. Palfree RGE, Hammerling U: Biochemical characterisation of the murine activated lymphocyte alloantigen Ly-6E.1 controlled by the Ly-6 locus. *J Immunol 136:594-600, 1986*

33. Hogarth PM, Houlden BA, Latham SE, Sutton VR, McKenzie IFC: Definition of new alloantigens encoded by genes in the Ly-6 complex.*Immunogenetics 20:57-69, 1984*

34. Flood PM, Murphy DB, Horowitz M, LeClair KP, Smith FR, Stockert E, Palladino MA Jr, DeLeo AB: A monoclonal antibody that recognises an Ly-6 linked antigen inhibits the generation of functionally active T cell subsets. *J Immunol 135:63-72, 1985*

35. Ortega G, Korty PE, Shevach EM, Malek TR: Role of Ly-6 in lymphocyte activation. I. Characterisation of a monoclonal antibody to a nonpolymorphic Ly-6 specificity. *J Immunol 137:3240-3246, 1986*

36. Rock KL, Yeh ETH, Gramm CF, Harber SI, Reiser H, Banacerraf B: TAP, a novel T cell-activating protein involved in the stimulation of MHC-restricted T lymphocytes. *J Exp Med 163:315-333, 1986*

37. Malek TR, Ortega G, Chan C, Kroczek RA, Shevach EM: Role of Ly-6 in lymphocyte activation. II. Induction of T cell activation by monoclonal anti-Ly-6 antibodies. *J Exp Med 164:709-722, 1986*

38. Leo O, Foo M, Segal DM, Shevach E, Bluestone JA: Selective expression of the Ly-6C.2 epitope on Lyt-2^+ T cells that can develop in the absence of thymic influence. *J Immunol 129:1214-1222, 1987*

39. Shevach EM: Accessory molecules. In: *Fundamental Immunology, Second Edition, 413-445, 1989*, Paul WE ed., Rowen Press, New York

40. Springer TA: Adhesion receptors of the immune system. Review Article. *Nature 346:425-434, 1990*

41. Sussmann JJ, Saito T, Shevach EM, Germain RN, Ashwell JD: Thy-1- and Ly-6-mediated lymphokine production and growth inhibition of a T cell hybridoma required co-expression of the T cell antigen receptor complex.

J Immunol 140:2520-2526, 1988

42. Yeh ETH, Reiser H, Bamezai A, Rock KL: TAP transcription and phosphatidylinositol linkage mutants are defective in activation through the T cell receptor. *Cell 52:665-674, 1988*

43. Flood PM, Dougherty JP, Ron Y: Inhibition of Ly-6A antigen expression prevents T cell activation. *J Exp Med 172:115-120, 1990*

44. Codias EK, Malek TR: Regulation of B lymphocyte responses to IL-4 and IFN-γ by activation through Ly-6A/E molecules: *J Immunol 144:2197-2204, 1990*

45. Thompson LF, Ruedi JM, Glass A, Lew JG, Lucas AH: Antibodies to 5'nucleotidase (CD73), a glycosylphosphatidylinositol-anchored protein, causes human peripheral blood T cells to proliferate. *J Immunol 143:1815-1821, 1989*

46. Davis LS, Patel SS, Atkinson JP, Lipsky PE: Decay-accelerating factor functions as a signal transfucing molecule for human T cells. *J Immunol 141:2246-2252, 1988*

47. Dumont FJ, Coker LZ: Interferon-α/β enhances the expression of Ly-6 antigens on T cells *in vivo* and *in vitro*. *Eur J Immunol 16:735-740, 1986*

48. Dumont FJ, Dijkmans R, Palfree RGE, Boldtz RD, Coker L: Selective upregulation by interferon-γ of surface molecules of the Ly-6 complex in resting T cells: the Ly-6A/E and TAP antigens are preferentially enhanced. *Eur J Immunol 17:1183-1191, 1987*

49. Jutila MA, Kroese FGM, Jutila KL, Stall AM, Fiering S, Herzenberg LA, Berg EL, Butcher EC: Ly-6C is a monocyte/macrophage and endothelial cell differentiation antigen regulated by interferon-gamma. *Eur J Immunol 18:1819-1826, 1988*

50. Halloran PF, Dutton D, Chance H, Cohen Z: An Ly-like specificity with extensive nonlymphoid expression. *Immunogenetics 7:185-200, 1978*

51. Van de Rijn M, Heimfeld S, Spangrude GJ, Weissman IL: Mouse hematpoietic stem-cell antigen Sca-1 is a member of the Ly-6 antigen family. *Proc Natl Acad Sci USA 86:4634-4638, 1989*

52. Dumont F, Coker L, Habbersett R, Treffinger J: Xenogeneic monoclonal antibody to an Ly-6 linked murine cell surface antigen differential reactivity with T cell subpopulations and bone marrow cells. *J Immunol 134:2357-65, 1985*

53. Herold KC, Montag AG, Meyer SM, Wojcikowski C, Fitch FW: Expression of Ly-6C by T lymphocytes of NOD mice after CD3-complex stimulation. Identification of activated cells during insulitis of prediabetic mice. *Diabetes 39:815-820, 1990*

54. Davies A, Simmons DL, Hale G, Harrison RA, Tighe H, Lachmann PJ, Waldman H: CD59, an Ly-6 like protein expressed in human lymphoid cells regulates the action of the complement membrane attack complex on homologous cells. *J Exp Med 170:637-654, 1989*

55. Sawada R, Ohashi K, Okano K, Hattori M, Minato N, Naruto M: Complementary DNA sequence and deduced peptide sequence for

CD59/MEM-43 antigen, the human homologue of murine lymphocyte antigen Ly-6C. *Nucleic Acids Res. 17:6728*

56. Okada H, Nagami Y, Takahashi K, Okada N, Hideshima T, Takizawa H, Kondo J: 20 KDa homologous restriction factor of complement resembles T cell activating protein. *Biochem Biophys Res Commun 163:1553-1559, 1989*

57. Bernard A, Horejsi V: Letter to the Editor. *J Immunol 143:1073, 1989*

58. Sawada R, Ohashi K, Anaguchi H, Okazaki H, Hattori M, Minato N, Naruto M: Isolation and expression of the full-length cDNA encoding CD59 antigen of human lymphocytes. *DNA and Cell Biol 9:213-220, 1990*

59. Holguin MH, Wilcox LA, Bernshaw NJ, Rosse WF, Parker CJ: Relationship between the membrane inhibitor of reactive lysis and the erythrocyte phenotypes of paroxysmal nocturnal haemoglobinuria. *J Clin Invest 84:1387-1394, 1989*

60. Okada N, Harada R, Fujita T, Okada H: A novel membrane glycoprotein capable of inhibiting membrane attack by homologous complement. *Int Immunol 1:205-208, 1989*

61. Sugita Y, Nakano Y, Tomitaq M: Isolation from human erythrocytes of a new membrane protein which inhibits the formation of complement transmembrane channels. *J Biochem 104:633-637, 1989*

62. Groux H, Huet S, Aubrit F, Tran HC, Boumsell L, Bernard A: A 19-kDa human erythrocyte molecule H19 is involved in rosettes, present on nucleated cells, and required for T cell activation. Comparison of the roles

of H19 and LFA-3 molecules in T cell activation. *J Immunol 142:3013-3020, 1989*

63. Meri S, Morgan BP, Davies A, Daniels RH, Olavesen MG, Waldmann H, Lachmann PJ: Human protectin (CD59), an 18,000-20,000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. *Immunology 71:1-9, 1990*

64. Rollins SA, Zhao J, Ninomiya H, Simms PJ: Inhibition of homologous complement by CD59 is mediated by a species-selective recognition conferred through binding to C8 within C5b-8 or C9 within C5b-9. *J Immunol 146:2345-2351, 1991*

65. Yamashina M, Ueda E, Kinoshita T, Takami T, Ojima A, Ono H, Tanaka H, Kondo N, Oril T, Okada N, Okada H, Inoue K, Kitani T: Inherited complete deficiency of 20-kilodalton homologous restriction factor (CD59) as a cause of paroxysmal nocturnal haemoglobinuria. *New Engl J Med 323:1184-1189, 1990*

66. Lublin DM, Atkinson JP: Decay-accelerating factor: biochemistry, molecular biology, and function. *Ann Rev Immunol 7:35-38, 1989*

67. Korty PE, Brando C, Shevach EM: CD59 functions as a signal transducing molecule for human T cell activation. *J Immunol 146:4092-4098, 1991*

68. Robinson PJ: Review: Phosphatidylinositol membrane anchors and T cell activation. *Immunol Today 12:35-41, 1991*

69. Philbrick WM, Palfree RGE, Maher SE, Bridgett MM, Serlin S, Bothwell ALM: The CD59 antigen is a structural homologue of murine Ly-6 antigens but

lacks interferon inducibility. *Eur J Immunol 20:87-92, 1990*

70. Meri S, Waldmann H, Lachmann PJ: Distribution of protectin (CD59), a complement membrane attack inhibitor, in normal human tissues. *Lab Invest 65:532-537, 1991*

71. Nose M, Katoh M, Okada N, Kyogoku M, Okada H: Tissue distribution of HRF20, a novel factor preventing the membrane attack of homologous complement, and its predominant expression on endothelial cells *in vivo*. *Immunology 70:145-149, 1990*

72. Rooney IA, Davies A, Griffiths D, Williams JD, Davies M, Meri S, Lachmann PJ, Nirgab BP: The complement-inhibiting protein, protectin (CD59 [illegible] present and functionally active on glomerular epithelial cells. [illegible]*unol 83:251-256, 1991*

[illegible]Walter EI, Rutgers JL, Knowles DM, Nussenzweig V: [illegible]the complement decay-accelerating factor (DAF) on [illegible]landular cells and in body fluids. *J Exp Med 165:848-864,*

74. Tamai H, Matsuo S, Fukatso A, Nishikawa K, Sakamoto N, Yoshioka K, Okada N, Okada H: Localization of 20-kd homologous restriction factor (HRF-20) in diseased human glomeruli. An immunofluorescence study. *Clin Exp Immunol 84:256-262, 1991*

75. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S: Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature 356:314-317, 1992*

76. Takei F: MALA-1: a surface antigen expressed on activated murine T and B lymphocytes. *J Immunol 133:345-350, 1984*

77. Auchincloss H, Ozato K, Sachs DH: Two distinct murine differentiation antigens determined by genes linked to the Ly-6 locus. *J Immunol 127:1839-1843, 1981*

78. Dijkema R, van der Meide PH, Pouwels PH, Caspers M, Dubbeld M, Schellekens H: Cloning and expression of the chromosomal, immune interferon gene of the rat. *EMBO J 4:761-767, 1985*

79. Jephthah-Ochola J, Urmson J, Farkas S, Halloran PF: Regulation of MHC expression *in vivo*. Bacterial lipopolysaccharide induces class I and II MHC products in mouse tissues by a T cell-independent, cyclosporine-sensitive mechanism. *J Immunol 141:792-800, 1988*

80. Halloran PF, Urmson J, van der Meid PH, Autenreid P: Regulation of MHC expression *in vivo*. II. IFN-α/β inducers and recombinant IFN-γ modulate MHC antigen expression in mouse tissues. *J Immunol 143:4241-4247, 1989*

81. Halloran PF, Autenried P, Ramassar V, Urmson J, Cockfield S: Local T cell responses induce widespread MHC expression. Evidence that interferon-γ induces its own expression in remote sites. *J Immunol 1992 (in press)*

82. Madrenas J, Parfrey NA, Halloran PF: Interferon gamma-mediated renal MHC expression in mercuric chloride-induced glomerulonephritis. *Kidney Int 39:273-281, 1991*

83. Jennette JC, Tidwell RR, Geratz JD, Bing DH, Falk RJ: Amelioration of immune complex-mediated glomerulonephritis by synthetic protease

inhibitors. *Am J Path 127:499-506, 1987*

84. Iskandar SS, Jennette JC, Wilkman AS, Becker RL: Interstrain variations in nephritogenicity of heterologous protein in mice. *Lab Invest 46:344-351, 1982*

85. Sambrook J, Fritsch EF, Maniatis T: Molecular cloning: a laboratory manual. *Cold Spring Harbor Laboratory Press 1989*

86. Wadgymar A, Urmson J, Baumal R, Halloran PF: Changes in Ia expression in mouse kidney during acute graft-vs-host disease. *J Immunol 132:1826-1830, 1984*

87. Skoskiewicz MJ, Colvin RB, Schneeberger EE, Russell PS: Widespread and selective induction of major histocompatibility complex determined antigens *in vivo* by gamma interferon. *J Exp Med 162:1645-1664*

88. Jevnikar AM, Wuthrich RP, Takei F, Hong-Wu X, Brennan DC, Glimcher LH, Rubin-Kelley VE: Differing regulation and function of ICAM-1 and class II antigens on renal tubular cells. *Kidney Int 38:417-425, 1990*

89. Wuthrich RP, Jevnikar AM, Takei F, Glimcher LH, Kelley VE: Intercellular adhesion molecule-1 (ICAM-1) expression is upregulated in autoimmune lupus nephritis. *Am J Path 136:441-450, 1990*

90. Lisanti MP, Sargiacomo M, Graeve L, Saltiel AR, Rodriguez-Boulan E: Polarized apical distribution of glycosyl-phosphatidylinositol-anchored proteins in a renal epithelial cell line. *Proc Natl Acad Sci USA 85:9557-9561, 1988*

91. Lisanti MP, Caras IW, Davitz MA, Rodriguez-Boulan E: A glycophospholipid anchor acts as an apical targeting signal in polarized epithelial cells. *J Cell Biol 109:2145-2156, 1989*

92. Brown DA, Crise B, Rose JK: Mechanism of membrane anchoring affects polarized expression of two proteins in MDCK cells. *Science 341:1499-1501, 1989*

93. Brown DA, Rose JK: Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell 68:533-544, 1992*

94. Cosio FG, Sedmak DD, Mahan JD, Nahman NS: Localization of decay accelerating factor in normal and diseased kidneys. *Kidney Int 36:100-107, 1989*

95. Gunter KC, Krpoczek RA, Shevach EM, Germain RN: Functional expression of the murine Thy-1.2 gene in transfected human T cells. *J Exp Med 163:285-292, 1986*

96. Hahn AB, Soloski MJ: Anti-Qa-2 induced T cell activation. The parameters of activation, the definition of mitogenic and nonmitogenic antibodies, and the differential effects of $CD4^+$ vs $CD8^+$ T cells. *J Immunol 143:407-413, 1989*

97. Appella E, Robinson EA, Ullrich SJ, Stoppelli MP, Corti A, Cassani G, Blasi F: The receptor-binding sequence of urokinase. *J Biol Chem 262:4437-4440, 1987*

98. Stefanova I, Horejsi V, Ansotegui IJ, Knapp W, Stockinger H: GPI-anchored

cell-surface molecules complexed to protein tyrosine kinases. *Science 254:1016-1019, 1991*

99. Pennica D, Kohr WJ, Kuang WJ, Glaister D, Aggarwal BB, Chen EY, Goeddel DV: Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein. *Science 236:83-88, 1987*

100. Paul LC, Baldwin WM, Van Es LA: Vascular endothelial alloantigens in renal transplantation. *Transplantation 40:117-123, 1985*

101. Menu E, Hahn WC, Bothwell AL, Sims PJ, Bierer BE: Overlapping but nonidentical binding sites on CD2 for CD58 and a second ligand, CD59. *FASEB J 6(4): A1224, 1992*

102. Korty PF, Tedder TF, Shevach EM: Identification of the ligands for the CD59 and Ly-6A.2 cell surface antigens. *FASEB J 6(4): A1224, 1992*

103. Cybulsky AV, Rennke HG, Feintzeig ID, Salant DJ: Complement-induced glomerular epithelial cell injury. Role of the membrane attack complex in rat membranous nephropathy. *J Clin Invest 77:1096-1107, 1986*

104. Yoshizawa N, Oshima S, Sagel I, Shimizu J, Treser G: Role of a streptococcal antigen in the pathogenesis of acute poststreptococcal glomerulonephritis. Characterisation of the antigen and a proposed mechanism for the disease. *J Immunol 148:3110-3116, 1992*