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CD52

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CD52 (Cambridge Pathology or more commonly Campath-1 antigen) is a low molecular weight glycoprotein with glycolipid-like properties present on the surface of most lymphocyte lineages, monocytes, and restricted cell types in the male reproductive system, specifically the epididymal epithelia. The peptide core is unusually small and contains a mere 12–amino acid residues, with a complex-class N-glycan attached to Asparagine 3 and a glycosylphosphatidylinositol (GPI)-anchor at the C-terminus (Serine 12) (see GPI-Anchored Proteins). Monoclonal antibodies directed against CD52 are very efficient at eliciting complement-mediated lysis and are useful for manipulation of lymphocyte levels in vivo and in vitro; hence considerable therapeutic interest in the molecule. CD52 is

also expressed in the male genital tract, and molecules are incorporated directly into sperm plasma membrane by direct insertion, where it appears to be a major component of the spermatozoa glycocalyx. The precise function of CD52, either in lymphocytes or in the reproductive tissue, is unknown at present, but engagement of CD52 with antibody demonstrates that the molecule can function as a signal transducer and elicit release of TNF-α (tumor necrosis factor-α), IFN-γ (interferon-γ), and (interleukin-6) IL-6.

PROTEIN CHEMISTRY

Immature CD52 polypeptides contain both N- and C-terminal signal sequences (Fig. 1) for targeting to the endoplasmic reticulum and for attachment of a GPI anchor, respectively. Apart from the extremely small size of the mature polypeptide chain at only 12–amino acid residues in humans, there is nothing known that is remarkable about CD52 processing and subsequent transport to the cell surface. The protein is expressed by lymphocytes, monocytes, and the epithelia lining the distal epididymal and deferent duct of the male genital tract, and carries a single highly processed N-glycan. The protein is sufficiently apolar to be extractable with methanol or Folch-type solvent systems. Significantly, the mature peptide backbone sequence is variable among different mammalian species, for example, being somewhat larger in the rat, giving rise to the view that the function of the peptide component is to provide a scaffold for the N-glycan and the GPI-anchor, and that the sequence of the peptide has no major role in CD52 function.

Both the N-glycan at Asn 3 and the GPI structures at Serine 12 are tissue-specific (Fig. 2). In splenic lymphocytes there are two populations of CD52, a CD52-I population that contains a disteararoylphosphatidylinositol GPI-anchor structure and a CD52-II population that bears a more conventional stearoylarachidonylphosphatidylinositol (1). Because of a palmitate esterified to the 2-position of the GPI inositol, CD52-II is resistant to phosphatidylinositol-specific phospholipase C (PI-PLC) cleavage. In contrast, seminal CD52 is 80% PI-PLC resistant, due to more extensive inositol palmitoylation, although this is a species-specific modification, as rat seminal CD52 is fully PI-PLC sensitive. An additional difference is that whereas the splenic GPI anchors are diacylglycerol forms, the seminal variant is *sn*-1-alkyl-2-*lyso* glycerol. This latter is a highly unusual GPI-anchor configuration in metazoans, but the functional implications of this structure remain unknown. There is no apparent novel modification or variation in the core GPI-glycan, but the lymphocyte form is elaborated with an extra mannose residue.

The N-glycan is probably functionally the most important component of CD52 based on current data. In lymphocytes the N-glycan is a highly processed tetraantennary structure, but again, male reproductive tract CD52 differs. In this tissue the number of reported glycoforms is more than 50, a remarkable level of heterogeneity of such a small molecule, and although triantennary structures are predominant, there is a significant proportion of larger species that bear extended lactosamine chains (2). Fucosylation of the lactosamines is significant and generates the VIM2 determinant, but importantly CD52 does not bear the sialyl-LewisX structure, which is a motif recognized by the selectin family of adhesion molecules. Both lymphocyte and epididymal forms are heavily sialylated, and thus contribute significantly to the net surface charge of the lymphocyte or spermatozoa.

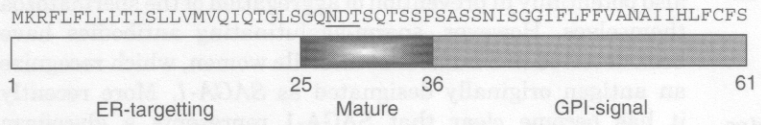

Figure 1. Amino acid sequence of human CD52 (Campath-1 antigen). Residues in the complete encoded protein are shown at the top and the N-glycosylation site is indicated by underline. The three functional domains (indicated at bottom) in the polypeptide are indicated in the ribbon in the center and their relative amino acid numbers shown.

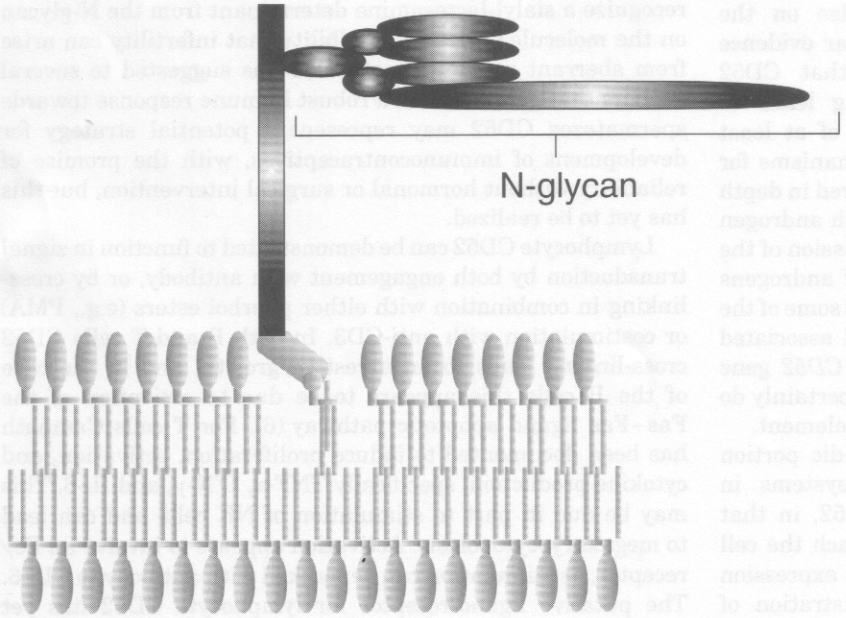

Figure 2. Graphic representation of lymphocyte CD52 (Campath-1 antigen). The image is drawn roughly to scale and depicts a single CD52 molecule embedded in the plasma membrane of a lymphocyte. Lipid molecules are represented by double-sticked lollipops. The peptide portion of CD52 is represented by the long vertical bar, whereas the N-glycan at Asn 3 is depicted as a collection of lozenges. In reality the molecule is likely to be highly flexible as the peptide portion is most probably devoid of a stable secondary structure.

MOLECULAR BIOLOGY

CD52 is expressed from a single-copy gene located at human chromosome 1p36.1 (3). Although there is a single gene for CD52, there is evidence for allelic polymorphism. The locus spans approximately 3 kbp, with the open reading frame split between two exons. Exon I encodes the first 18 residues of the signal sequence, and exon II encodes the remainder of the peptidic portion of the molecule, including the C-terminal GPI signal. Most significantly there are multiple mRNA initiation sites mapping to the −60 bp region from the ATG start codon. The promoter is devoid of a conventional TFIID TATA box sequence, despite being shown to be a functional promoter element in heterologous reporter systems. There is also heterogeneity in polyadenylation, and some evidence suggests that this is functionally important in the epididymal form. Specifically epididymal epithelia express two major CD52 mRNA species, short and long variants, which are differentially regulated; expression of the short mRNA is influenced by androgen steroid hormones, whereas the long form is strongly attenuated by increased temperature (4).

The most similar molecule so far identified to CD52 is another lymphocyte antigen, CD24. This latter molecule is expressed on B cells and influences signal transduction. For CD24 the mature protein is 31 to 35 amino acids in length, bears an N-glycan, and is anchored by a GPI moiety. There are also clear CD52 homologs in other mammalian species, and in mouse the B7-Ag most probably corresponds to human CD52. Strikingly there is little conservation of the sequence of the mature peptide, but both the ER-targeting and GPI-addition signal sequences are highly conserved. In addition the B7-Ag locus is very similar in structure to the human CD52 locus (3). These data may be taken to suggest that the peptidic portion is important primarily for the attachment of the N-glycan to the GPI-anchor moiety, and hence the plasma membrane, and hence is unlikely to have a role beyond the purely structural.

CELL BIOLOGY

CD52 is expressed in two major lineages; lymphocytes, including most B and T cells, eosinophils, possibly natural killer (NK) cells, but not neutrophils, and also on the epididymal epithelia. In lymphocytes there is clear evidence from bivalent antibody cross-linking studies that CD52 is capable of signal transduction. Cross-linking leads to lymphocyte proliferation and also to production of at least three cytokines, TNF-α, IFN-γ, and IL-6 (5). Mechanisms for regulation of the level of CD52 have not been explored in depth in lymphocytes, but by contrast it is clear that both androgen levels and temperature affect the epididymal expression of the molecule. Elevated temperature or withdrawal of androgens lead to decreased expression, accounting for at least some of the restricted expression profile within the testis and associated structures. However, the control elements in the *CD52* gene promoter have not been functionally mapped, and certainly do not appear to contain a regular steroid-responsive element.

Despite the extremely small size of the peptidic portion of the molecule, the normal quality control systems in the secretory pathway appear to operate on CD52, in that compromised processing results in a failure to reach the cell surface. Specifically, in a number of studies CD52 expression has been experimentally suppressed by administration of antibody therapeutics (see following section), and this leads to the appearance of lymphocytes devoid of all GPI-anchored marker proteins, a condition that resembles paroxysmal nocturnal hemoglobinuria (PNH), a hemolytic disorder due to an inability to inactivate complement components on the erythrocyte surface (6,7). Under most circumstances PNH is primarily due to a somatic mutation in the X-linked *PIG-A* gene, and hence present in single-gene dosage in both males and females. The product of the *PIG-A* gene is essential for an early step in the synthesis of the GPI-anchor precursor, and in PNH these metabolites are not found in affected cells. Absence of the GPI-anchor normally leads to a failure in surface expression of GPI proteins and retention by the intracellular quality control system (8), and apparently CD52 is no exception despite the small size of the molecule.

There is no evidence that sperm or their progenitors express CD52 mRNA; hence all the molecules that are found on the plasma membrane of mature spermatozoa are presumed to have been acquired from the epididymal epithelia via a shedding and reincorporation process. The function of CD52 in the epididymal epithelia has not been investigated and is not known. There is precedent for this type of migratory behavior from observations of other GPI-linked proteins. The CD52 molecules are not restricted to a particular region of the sperm plasma membrane, which is also consistent with this mode of acquisition as GPI-anchored proteins are frequently polarized in their surface expression, and endogenously synthesized GPI-anchored proteins are found predominantly in the sperm head region. There is evidence for differential antibody reactivity for the CD52 present on the head region and tail of human spermatozoa, but the structural basis for this is not known at present (9). No direct function has been ascribed to sperm CD52. The presence of highly sialylated and bulky glycans on sperm CD52 may suggest a passive role in suppression of immune recognition of surface epitopes on other molecules, and also potentially in prevention of aggregation of the spermatozoa themselves. However, sperm-agglutinating antibodies have been detected in a number of infertile women, which recognize an antigen originally designated as *SAGA-1*. More recently it has become clear that SAGA-1 represents a glycoform subpopulation of CD52 and that many of these antibodies recognize a sialyl-lactosamine determinant from the N-glycan on the molecule (10). The possibility that infertility can arise from aberrant recognition of CD52 has suggested to several authors that generation of a robust immune response towards spermatozoa CD52 may represent a potential strategy for development of immunocontraceptives, with the promise of reliability without hormonal or surgical intervention, but this has yet to be realized.

Lymphocyte CD52 can be demonstrated to function in signal transduction by both engagement with antibody, or by cross-linking in combination with either phorbol esters (e.g., PMA) or costimulation with anti-CD3. In both B and T cells CD52 cross-linking leads to an arrest in growth, and in the case of the B cells this appears to be due to activation of the Fas–Fas ligand apoptotic pathway (6). For T cells Campath has been documented to induce proliferation, activation, and cytokine production, specifically TNF-α, IFN-γ, and IL-6. This may be due in part to stimulation of NK cells and can lead to megakaryocytopoiesis. Activation appears to involve an Fcγ receptor, as stimulation can be blocked with antibody to CD16. The putative ligand/receptor for lymphocyte CD52 has yet

to be identified. Given the prominence of the N-glycan, it is plausible that recognition of the N-glycan via a mammalian lectin is an important facet of CD52 function, but this remains to be demonstrated.

THERAPEUTIC UTILITY

Interest in CD52 initially arose from the finding that antibodies directed against this antigen were exquisitely powerful at mediating complement-dependant lysis (11). As with the sole exception of the male reproductive tract, CD52 expression appears confined to cells of the lymphocyte lineage, and this finding has clear implications for potential clinical intervention in numerous diseases where lymphocyte levels are either pathologic, as obviously in leukemias or where there is a requirement for experimental manipulation of the lymphocyte compartment, for example, for temporary or even permanent ablation of the immune response.

The original rat monoclonal antibodies raised against CD52 were designated as Campath-1G and Campath-1M, reflecting their isotypes as an IgG and IgM, respectively. Subsequently, Campath-1G was "humanized," that is, the nucleotide sequences encoding the complementarity determining regions were grafted into a human IgG sequence by recombinant DNA techniques, to create Campath-1H (see HUMANIZING ANTIBODIES). This chimeric antibody is the form that has been used in the vast majority of studies on the utility of CD52 for lymphocyte ablation, and has the advantage that the production of an immune response against the nonhuman sequences are minimized. X-ray crystallography has demonstrated that the interactions between CD52 and Campath-1H and Campath-1G at the atomic level are extremely similar. Because there are major isotype-specific differences in complement activation and other functions the different forms of Campath may potentially be optimal in different applications (see the following text).

MANIPULATION OF LYMPHOCYTES WITH CAMPATH

The various Campath antibodies have been used as components of protocols in several thousand clinical studies for both in vivo and in vitro depletion of lymphocytes over the last decade. Applications have included treatment of bone marrow grafts to reduce the incidence of graft-versus-host disease (GVHD), ablation of lymphocytes in various leukemias, and also several attempts to treat autoimmune conditions, for example, rheumatoid arthritis (RA) and multiple sclerosis (MS). Some of these applications show promise in the clinical setting, with the major utility ultimately being an adjunct to other existing procedures, and hence complementing either chemotherapy or immunosuppression. In addition a number of potentially serious sequelae have been observed (see next section), which may limit the use of CD52 in the clinical arena.

Campath-1M is particularly useful for the depletion of T cells from bone marrow for matched or related bone marrow transplantation (BMT), and does decrease GVHD. Campath-1G is most used for the ablation of lymphocytes in vivo by intravenous injection to treat lymphomas and attenuate kidney graft rejection episodes. In addition the antibody is useful for attenuating rejection in BMT from unrelated doors. The humanized form, Campath-1H, is mainly used in treating organ graft rejection, and for reduction of lymphocyte populations to treat lymphoma or leukemia, and is being commercialized.

The use of CD52 ablation in bone marrow transplantation BMT for treatment of leukemias and other pathologies has had mixed success. The particular promise from Campath antibody therapy in this arena lies mainly in the ability to remove both the B- and T-cell populations with a single procedure. As more traditional methods mainly lead to depletion of just T cells, there is potential for abnormal and dangerous B-cell proliferation. However, T-cell depletion can by itself lead to increased graft rejection, possibly because of loss of suppressor compartments. In a recent study where BMT was used to treat acute lymphoblastic leukemia (ALL), there was a significant decrease in the prevalence of GVHD following Campath-mediated depletion of lymphocytes from the graft (12). In addition a combination of Campath-1M for in vitro depletion of grafts, and Campath-1G treatment of the recipients achieved a significant improvement of engraftment rates (13). However, in a long-term follow-up study of BMT-treated ALL patients, although Campath-1H clearly improved the efficacy of engraftment and decreased GVHD, there was no significant long-term benefit over a five-year period.

Treatment of leukemias in vivo has also been attempted using Campath antibodies. There are numerous studies, but the two most recent reports present particularly encouraging data. In the first study Campath-1H was used to treat patients with T-cell proliferative leukemia. The leukemias were aggressive and resistant to purine analog chemotherapy. Medium-term benefit was clearly obtained, but no improvement in long-term remission (14). In the second study Campath-1H was used to effect successful remove of residual lymphocytes after conventional chemical treatments from chronic lymphocytic leukemia patients (15).

Other lymphoproliferative diseases have also been experimentally treated with antibodies against CD52. These include Wegener's granulomatosis (WG) and low grade non-Hodgkin's lymphoma (16,17). For WG, Campath-1H therapy was impressive, with a more rapid response and lower toxicity than conventional therapies, but the data from the non-Hodgkin's lymphoma study were less encouraging, and antibody therapy had only a small beneficial effect. With this latter study and some of the examples of leukemias discussed above, it is possible that Campath treatment is actually significantly better than suggested by these trials, as in most cases the disease is already refractory to conventional chemotherapies, which is known to result frequently in large phenotypic alterations to the remaining population of transformed lymphocytes.

There have been a few attempts to treat MS and RA with Campath antibodies. These studies are small and report some remission. However, significant complications are also evident from these studies, so that the significance of any benefit must be evaluated against this background. Data do not indicate that Campath offers a significant advantage over high-quality conventional therapies for these diseases at present.

LIMITATIONS AND ADDITIONAL EFFECTS

Emergence of a GPI-negative lymphocyte subset following Campath antibody treatment has been independently observed on several occasions, usually in a minority of patients in the study group (18). A simple interpretation of these data is that

there is a huge selective pressure in favor of a small self-renewing population of endogenous PNH cells that populate the lymphocyte compartment, but this is insufficient to explain fully all of the data. In one example following recovery from lymphocyte ablation, T-cell lines have been isolated from RA patients that are 10% CD52 positive and 90% negative. A simple mutation in the coding sequence for PIG-A can be ruled out, as culture of the positive or negative population following cell sorting results in reappearance of the biphasic CD52 expression distribution at a frequency that cannot be accounted for by a simple genetic reversion. Further, injection of CD52-negative cells into mice augments CD52 expression, and a defect in the PIG-A gene was undetectable (7). This suggests that a more complex but entirely unknown mechanism accounts for the resultant PNH-like population, which may be difficult to control.

A further potential difficulty has been highlighted mainly from studies in MS. Here the possibility of ameliorating the condition by depleting activated lymphocytes from the central nervous system is complicated by the ability of CD52 to activate lymphocytes; this can lead to the reawakening of preexisting symptoms (19). The effect is short-lived but certainly is consistent with transient production of proinflammatory cytokines. In addition, some MS patients have experienced lymphopenia following Campath administration. Finally, there is also suggestion that there is a significant increase in infection in patients following Campath therapy. It is not yet clear if this is a result of the resultant immunosuppression or of the procedure itself.

Long-term abnormalities in lymphocyte populations following Campath treatment have also been reported. The T-cell compartment appears the most sensitive, and in one study the CD4+ population did not return to normal even after a >500 day follow-up period. Additionally the first population of lymphocytes to repopulate the blood carried activation markers (20).

CONCLUSION

The CD52 molecule, expressed on lymphocytes and epididymal epithelia, remains without documented function. It is a member of the GPI-anchored protein family, but the peptide moiety is so small that it resembles more a complex glycolipid. Evidence demonstrates that CD52 can function as a signaling molecule, but it remains unclear if this reflects a physiologically important role in the absence of a defined ligand. Extensive tissue-specific glycosylation has been documented, and in the case of the form that occurs on sperm it is believed that the glycan has an important role in evasion of the immune system.

Interest in CD52 stems from the ability to exploit it for lymphocyte depletion by antibody-mediated complement lysis. As a therapeutic procedure there are some encouraging data. In manipulation of bone marrow grafts anti-CD52-mediated lymphocyte ablation has clear medium-term benefit. Similarly for treatment of lymphoproliferative diseases directly, this novel therapy shows promise, particularly when used in combination with other regimes. Complications clearly exist, and most successful outcomes have been achieved with careful monitoring and subsidiary therapies. It remains to be determined which lymphoproliferative diseases, and which strategies of treatment, will benefit most from CD52-depletion protocols.

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ADDITIONAL READING

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