Take-home messages

Bullet point: Mannose binding lectin (MBL) is a serum protein important in innate immunity and is protective against various infective organisms.

Bullet point: MBL binds carbohydrates on various infectious agents, and has an opsonin effect. In addition, it activates the lectin pathway of the complement cascade.

Bullet point: There is a large inter-individual difference in the serum concentration of MBL, caused mainly by MBL gene polymorphisms (SNP). Individuals with the minority alleles have lower serum MBL concentration.

Bullet point: Individuals homozygous for the minority allele of the MBL gene have higher risk of infection during infancy and when under immunosuppressive therapies.

Bullet point: Individuals homozygous for the minority allele of the MBL gene seem to be at a higher risk of acquiring autoimmune diseases, systemic lupus erythematosus (SLE) in particular.

Bullet point: Autoantibodies against MBL are present in some SLE patients, but their significance remains unclear.

Introduction

Mannose binding lectin (MBL) is a serum protein produced in the liver, and is a key molecule in innate immunity. MBL, along with other molecules such as surfactant protein A (SP-A) and surfactant protein D (SP-D), is a member of the collectin family, the characteristic of which being possession of a carbohydrate recognition domain (CRD) and a collagenous domain [1].

MBL is a trimer protein composed of 3 identical polypeptides with a molecular weight of around 32Kd (228 amino acids), and 3 to 6 trimers further combine to make a huge bouquet-like structure similar to that of complement C1q [2] (Figure 1-A). Each polypeptide consists of a CRD, a neck domain, a collagen domain and a cystein rich region. MBL binds to various organisms by its CRD, and excises an opsonin effect. The multimeric structure of MBL allows it to bind to various microorganisms including gram positive and negative bacteria, mycobacterium, viruses and fungi. The binding of MBL leads to agglutination of these microorganisms and will help their clearance by phagocytes. In addition, MBL activates the complement pathway (the lectin pathway) through mannose binding lectin associated serine proteases (MASPs). Therefore, MBL is important in host defense, especially in infancy, when the acquired immunity has not fully developed. Individuals lacking this protein could develop severe episodes of bacterial infections from early life [3,4]. MBL is also important when the immune system of an individual is compromised, such as when a patient is under immunosuppressive therapy, or receiving chemotherapies or bone marrow transplant [5].

While MBL is an acute phase protein and its production is enhanced by inflammatory stimuli, polymorphisms of the MBL gene is known to greatly influence serum MBL concentration. The MBL gene, located on the long arm of chromosome 10 at 10q11.2-q21, contains 4 exons [2]. There are 5 known single nucleotide polymorphisms (SNPs) that affect serum MBL concentration [6-9] (figure 1-B). Codon 52 (+223), 54(+230) and 57(+239) polymorphisms are all on exon 1, and the minority alleles are designated allele D, B, C, respectively, while the majority allele is designated allele A. Presence of any of the minority alleles (collectively designated allele O) results in an amino acid substitution and significant reduction of serum MBL concentration. Furthermore, homozygosity

for the minority alleles (genotype OO) results in almost complete deficiency of serum MBL [6,7]. This has been attributed to increased degradation of the mutated protein [7]. Frequencies of the minority alleles differ significantly among ethnicities, varying from around 1% to up to around 30 %. In the promoter region of the MBL gene, polymorphisms are reported at positions -550, -221 and +4, and these polymorphisms also influence the levels of serum MBL [9,10] (Table 1). Thus, some individuals with MBL genotype AO or even AA may have extremely low serum MBL concentration. In this review, however, we will focus on the SNPs in the coding region of the MBL gene.

Mannose binding lectin and autoimmune diseases

A number of studies have suggested that MBL deficiency, or low serum MBL levels caused by the SNPs described above may be associated with occurrence of SLE [11]. By a meta analysis of 8 previous studies, it has been shown that presence of the minority alleles (B, C or D alleles) confer a 1.6 times overall increased risk of acquiring SLE [11]. In the same study, by observing their own patients, Garred et al reported that the lag time from the appearance of the first lupus attributable symptom to the diagnosis of definite SLE was shorter in patients carrying at least one minority allele than in patients homozygous for the majority allele [11]. Two possible explanations for the associations between MBL deficiency and the occurrence of SLE are suggested (Figure 1-C). Firstly, MBL can bind to and initiate uptake of apoptotic cells by macrophages [12], and an abnormal accumulation of cell debris would occur in MBL deficient individuals, and would serve as a source of autoantigens. In accord with this hypothesis, Seelen et al [13] recently reported that anticardiolipin and anti-C1q antibodies were observed significantly more frequently in SLE patients with MBL minority alleles than those without those alleles, and that presence of those antibodies were associated with decreased serum MBL concentration and function. It is also reported that MBL can bind DNA, and MBL may have a role in clearance of DNA [14]. Secondary, since MBL has a major role in innate immunity, individuals with MBL deficiency might have higher possibilities of being infected with pathogens that have some roles in the pathogenesis of SLE. However, these hypotheses are yet to be proved. The recent production of MBL deficient mice

[16] may shed a light on the relationship between MBL deficiency and autoimmune diseases.

Regardless of the mechanisms involved, MBL deficiency by itself is not sufficient to cause SLE. Unlike C1q deficiency, which is very strongly associated with lupus or lupus like syndromes [16-21], the majority of individuals with MBL genotype OO do not acquire SLE or any other autoimmune diseases, and are not significantly vulnerable to infectious agents when they are healthy and mature. Some other factors must be necessary for individuals with MBL genotype OO individuals to acquire SLE. What those factors are is still unknown.

While the association between MBL deficiency and SLE suggests that MBL has protective functions against occurrence of autoimmune diseases, deposition of MBL in kidneys of SLE patients [22] and glands of Sjogren's syndrome patients [23] have been reported. MBL may bind to carbohydrates of various proteins and may activate the lectin pathway of the complement pathway. From this point of view, MBL may have a role in the development of tissue damage in these diseases. Thus, MBL may be a double-edged blade in autoimmune diseases. This is also true for the role of MBL in infections. MBL is reported to enhance the progression of some infectious diseases [24], and in this situation, MBL deficiency can be beneficial. The relatively large prevalence of MBL deficiency in the general population also suggests that MBL deficiency may be advantageous for survival in certain conditions.

How MBL and the lectin pathway of the complement activation cascade affect the clinical course of SLE is not well known. It has been shown than a weak but significant positive relationship exist between serum MBL concentration and CH50, suggesting that the serum levels of MBL are associated with the disease activity of SLE in some way [25]. In this study, Takahashi et al also studied the time course of serum MBL concentration in newly diagnosed patients. In SLE patients with MBL genotype AA, serum MBL concentrations did fluctuate during the course of the disease, but there was no clear trend common to all patients. In some patients, serum MBL concentration decreased after initiation of immunosuppressive therapy, while in others serum MBL concentration increased in parallel with CH50 values. Serum MBL levels

are determined by a balance of production and consumption. Since MBL is an acute phase protein, MBL production may be increased in SLE patients with severe inflammation, while deposition of MBL to various tissues may lower its serum concentration. The levels of increase or deposition to tissues would differ among individual patients. Thus, most probably, concentration of serum MBL cannot be simply interpreted to SLE disease activity or an involvement of a particular organ.

Association between MBL and rheumatoid arthritis (RA) has also been suggested. It has been suggested that MBL may bind IgG with altered glycosylation and thus may be pathogenic in patients with RA [26]. However, from the results of clinical studies, it seems that RA patients with MBL genotypes OO show more rapid progression of joint destruction than those without this genotype [27]. If this finding could be confirmed by additional studies, typing of the MBL genotypes may become of value to identify RA patients with a higher risk of rapid joint destruction, and will aid in the selection of therapies for individual RA patients.

Few reports on the relationships between MBL gene polymorphisms and other autoimmune diseases exist. Mullighan et al [28] reported that there is no association between occurrence of Sjogren's syndrome and MBL genotypes. Tsutsumi et al reported that MBL deficiency may be a minor risk factor for the occurrence of type I diabetes [29]. Recently, we examined the MBL genotypes in 53 patients with mixed connective tissue disease (MCTD). Among these patients, only 1 patient had genotype BB (Tsutsumi et al, unpublished observations). Thus, unlike SLE, MBL genotype OO do not seem to be a risk factor for having MCTD.

MBL and vascular disorders

MBL also may be implicated in vascular diseases in both the general population and in autoimmune disease patients. It is reported that MBL enhances tissue injury caused by reperfusion, and administration of anti-MBL antibodies ameliorates myocardial ischemia-reperfusion injury in rat models [30]. In addition, MBL mediated complement activation has been reported to be important in mice renal ischemia-reperfusion injury model [31]. On the other

hand, MBL deficiency is reported to be associated with enhanced atherosclerosis [32] and coronary artery disease [33] in humans. In addition, a recent report indicated a strong association between the OO genotype of the MBL gene and occurrence of arterial thromboses in patients with SLE [34]. In this study, genotypes of the MBL gene were investigated in 91 patients with SLE. Among their 7 patients with MBL genotype OO, 6 had history of arterial thromboses, while 18 of SLE patients with MBL genotypes AA or AO had such history. They concluded that having an OO genotype of the MBL gene may be a major risk factor of having arterial thrombosis in patients with SLE. Several explanations for this association are possible. Possession of genotype OO may be associated with higher disease of SLE, which may enhance vascular injuries, and may necessitate larger amount of steroid for therapy. Alternatively, possession of genotype OO, and hence being deficient of functional MBL may render those SLE patients more susceptible to microorganisms associated with atherosclerosis. It has been suggested that *Chlamydia Pneumoniae* infection is related with occurrence of coronary heart diseases. Furthermore, it has been shown that individuals with MBL gene O alleles, and are positive for serum anti *Chlamydia Pneumoniae* antibodies may be more likely to have coronary artery disease [35]. *Chlamydia Pneumoniae* infection may enhance the development of coronary heart disease in SLE patients lacking serum MBL. To date, there is no direct evidence that this is indeed the case. If future studies confirm the association between MBL gene O alleles and arterial thromboses, it may become possible to identify SLE patients at a higher risk of having arterial thrombosis at the time of diagnosis. Taking appropriate protective measures would aid in the improvement of the prognosis of SLE patients. As the number of SLE patients with MBL genotype OO was not very large in this study, this observation need to be confirmed by future studies with a larger study population.

The association between MBL genotypes of SLE patients and infection has been suggested [11,25,36]. Since the mainstream of therapy for SLE is immunosuppression, typing the MBL gene or measuring serum MBL before immunosuppressive therapies may aid in assessing the risk of infection during such therapies.

Autoantibodies against mannose binding lectin

It is well established that autoantibodies against complement C1q is found in 30-45% of SLE patients, and the presence of anti-C1q antibodies is associated with glomerulonephritis and hypocomplementemia. The relationship between C1q deficiency and SLE like symptoms is also well known. The structural and functional similarity between C1q and MBL, and the large differences of serum MBL concentration among individuals with the same MBL genotype prompted some investigators to search for the presence of anti-MBL autoantibodies (anti-MBL). Anti-MBL, if present, may bind to MBL and decrease its serum concentration. Alternatively, anti-MBL may bind to MBL already deposited in various tissues and enhance tissue injury.

Seelen et al reported that anti-MBL were indeed present in sera of some patients with SLE [37]. In addition, they found that anti-MBL is present as a complex with circulating MBL, and that an inverse relationship exists between titer of anti-MBL and the functional activity of MBL.

Takahashi et al found elevated anti-MBL levels in sera 9 of 111 SLE patients, compared to 2 of 113 healthy controls [38]. There was no significant relationship between the levels of anti-MBL and serum MBL concentration. Interestingly, not only subjects with MBL genotype AA, but also some subjects with genotype AB had elevated anti-MBL activity. They were not able to link the presence of anti-MBL to clinical parameters or features of SLE, including malar rash, photosensitivity, arthritis, serositis, renal disorders, neurological disorders, hematologic disorders or titers of other commonly measured autoantibodies such as anti-nuclear antibody, anti-DNA antibody, anti-Sm antibody and antiphospholipid antibody. In addition, no apparent relationship between positivity of anti-MBL and episode of infection was noticed. However, as only 9 patients were positive for anti-MBL in this study, it is too early to conclude that anti-MBL do not have any clinical significance.

A recent study by Mok et al also searched for anti-MBL in sera of SLE patients. In their cohort of 135 SLE patients, 32(23.7%) were positive for IgG anti-MBL [39]. They also reported the presence of IgM class anti-MBL in a small fraction of SLE patients. Differing from the study by Takahashi et al, they

noticed a significant positive relationship between the levels of serum MBL and titers of IgG anti-MBL, in patients positive for anti-MBL. However, similar to the study by Takahashi et al, they were also unable to find any relationships between serum anti-MBL levels and various parameters including overall disease activity, alopecia, cerebral involvement, autoimmune hemolytic anemia, oral ulceration, photosensitivity, polyarthralgia, renal involvement, serositis, skin rash, thrombocytopenia, and autoantibodies such as antinuclear antibody, anti-Sm, anti-RNP, anti-SS-A, anti-SS-B and anti-DNA antibodies.

The differences in the prevalence of anti-MBL in the studies by Takahashi et al [38] and Mok et al [39] may partly explained by the cut off levels used by these studies. While the study by Takahashi et al used mean + 2SD of values from healthy individuals, the study by Mok et al used 90 percentile of values from healthy subjects. If the cut off level for anti-MBL positivity was set at 90 percentile of values from healthy subjects in the study by Takahashi et al, the prevalence of anti-MBL in SLE patients would have been similar to that obtained in the study by Mok et al. At this stage, the prevalence and significance of anti-MBL autoantibody in patients with SLE still remains obscure.

Future prospective

A number of studies have established the relationship between the OO genotypes of the MBL gene and SLE. However, the mechanism by which this association occur is not clearly elucidated. In addition, The relationship between serum MBL levels and occurrence, progression and complications of SLE, and the value of serum MBL measurement in a clinical setting has not been clearly established. A cohort study in a larger scale is necessary to determine the clinical value of MBL gene typing or serum MBL measurement in a clinical setting. Mbl typing seems to be more promising at this time, but measurement of serum MBL by enzyme immunoassays would be more convenient. The significance of fluctuation in the levels of serum MBL in individual SLE patients is also still unclear. Whether MBL deficiency or presence of anti-MBL is associated with various complication of SLE, or with particular vascular involvements should also be addressed in a large cohort of patients. A clear knowledge of these issues may aid in improving the prognosis of SLE patients.

Furthermore, solid understanding of the role of MBL in various conditions will aid to assess whether some patients will benefit from MBL replacement, or measures to inhibit the action of MBL.

References

[1] Holmskov U, Malhotra R, Sim RB, Jensenius JC. Collectins: collagenous C-type lectins of the innate immune defense system. Immunology Today 1994;15:67-74.

[2] Sastry K, Herman GA, Day L , Deignan E, Bruns G, Morton CC, Ezekowitz RA. The human mannose-binding protein gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localization to chromosome 10. J Exp Med 1989;170:1175-1189.

[3] Koch A, Melbye M, Sorensen P, Homoe P, Madsen HO, Molbak K, Hansen CH, Andersen LH, Hahn GW, Garred P. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA 2001;285:1316-1321.

[4] Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. BMJ 1997;314:1229-1232.

[5] Mullighan CG, Bardy PG. Mannose-binding lectin and infection following allogeneic hemopoietic stem cell transplantation. Leuk Lymphoma. 2004 45:247-56.

[6] Madsen HO, Garred P, Kurtzhals JA, Lamm LU, Ryder LP, Thiel S, Svejgaard A. A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. Immunogenetics 1994;40:37-44.

[7] Sumiya M, Super M, Tabona P Levinsky RJ, Arai T, Turner MW, Summerfield JA. Molecular basis of opsonic defect in immunodeficient children. Lancet 1991;337:1569-1570.

[8] Lipscombe RJ, Sumiya M, Hill AV, Lau YL, Levinsky RJ, Summerfield JA, Turner MW. High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. Hum Mol Genet 1992;1:709-715.

[9] Madsen HO, Satz ML, Hogh B, Svejgaard A, Garred P. Different molecular

events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America. J Immunol 1998;161:3169-175.
[10] Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol. 1995;155:3013-3020.
[11] Garred P, Voss A, Madsen HO, Junker P. Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. Genes Immun 2001;2:442-450.
[12] Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, Henson PM. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med. 2001;194:781-795.
[13] Seelen MA, van der Bijl EA, Trouw LA, Zuiverloon TC, Munoz JR, Fallaux-van den Houten FC, Schlagwein N, Daha MR, Huizinga TW, Roos A. A role for mannose-binding lectin dysfunction in generation of autoantibodies in systemic lupus erythematosus. Rheumatology (Oxford) 2005;44:111-119.
[14] Palaniyar N, Nadesalingam J, Clark H, Shih MJ, Dodds AW, Reid KB.Nucleic acid is a novel ligand for innate, immune pattern recognition collectins surfactant proteins A and D and mannose-binding lectin.
J Biol Chem 2004;279:32728-32736.
[15] Shi L, Takahashi K, Dundee J, Shahroor-Karni S, Thiel S, Jensenius JC, Gad F, Hamblin MR, Sastry KN, Ezekowitz RA. Mannose-binding lectin-deficient mice are susceptible to infection with Staphylococcus aureus. J Exp Med. 2004;199:1379-1390.
[16] Nishino H, Shibuya K, Nishida Y, Mushimoto M. Lupus erythematosus-like syndrome with selective complete deficiency of C1q. Ann Intern Med 1981;95:322-324.
[17] Kirschfink M, Petry F, Khirwadkar K, Wigand R, Kaltwasser JP, Loos M. Complete functional C1q deficiency associated with systemic lupus erythematosus (SLE). Clin Exp Immunol 1993;94:267-272.
[18] Bowness P, Davies KA, Norsworthy PJ et al. Hereditary C1q deficiency

and systemic lupus erythematosus. QJM 1994;87:455-464.
[19] Walport MJ, Davies KA, Botto M. C1q and systemic lupus erythematosus. Immunobiology 1998;199:265-285.
[20] Berkel AI, Petry F, Sanal O et al. Development of systemic lupus erythematosus in a patient with selective complete C1q deficiency. Eur J Pediatr 1997;156:113-115.
[21] Slingsby JH, Norsworthy P, Pearce G et al. Homozygous hereditary C1q deficiency and systemic lupus erythematosus. A new family and the molecular basis of C1q deficiency in three families. Arthritis Rheum 1996;39:663-670.
[22] Hisano S, Matsushita M, Fujita T, Endo Y, Takebayashi S. Mesangial IgA2 deposits and lectin pathway-mediated complement activation in IgA glomerulonephritis. Am J Kidney Dis 2001;38:1082-1088.
[23] Steinfeld S, Penaloza A, Ribai P, Decaestecker C, Danguy A, Gabius HJ, Salmon I, Appelboom T, Kiss R.D-mannose and N-acetylglucosamine moieties and their respective binding sites in salivary glands of Sjogren's syndrome. J Rheumatol. 1999;26:833-841.
[24] Santos I K, Costa C H, Krieger H, Feitosa M F, Zurakowski D, Fardin B, Gomes R B, Weiner D L, Harn D A, Ezekowitz R A, Epstein J E. Mannan-binding lectin enhances susceptibility to visceral leishmaniasis. Infect Immunol 2001;69:5212-5215.
[25] Takahashi R, Tsutsumi A, Ohtani K, Muraki Y, Goto D, Matsumoto I, Wakamiya N, Sumida T. Association of mannose binding lectin (MBL) gene polymorphism and serum MBL concentration with characteristics and progression of systemic lupus erythematosus. Ann Rheum Dis 2005;64:311-314.
[26] Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of Ig associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nature Med 1995;1:237-243.
[27] Graudal NA, Madsen HO, Tarp U, Svejgaard A, Jurik G, Graudal HK. The association of variant mannose-binding lectin genotypes with radiographic outcome in rheumatoid arthritis. Arthritis Rheum 2000;43:515-521.
[28] Mullighan CG, Heatley S, Bardy PG et al. Lack of association between mannose-binding lectin gene polymorphisms and primary Sjogren's syndrome. Arthritis Rheum. 2000;43:2851-2852.

[29] Tsutsumi A, Ikegami H, Takahashi R, Murata H, Goto D, Matsumoto I, Fujisawa T, Sumida T. Mannose binding lectin gene polymorphism in patients with type I diabetes. Hum Immunol 2003;64:621-624.
[30] Jordan JE, Montalto MC, Stahl GL. Inhibition of mannose-binding lectin reduces postischemic myocardial reperfusion injury. Circulation. 2001 Sep 18;104:1413-1418.
[31] de Vries B, Walter SJ, Peutz-Kootstra CJ, Wolfs TG, van Heurn LW, Buurman WA. The mannose-binding lectin-pathway is involved in complement activation in the course of renal ischemia-reperfusion injury. Am J Pathol 2004;165:1677-1688.
[32] Madsen HO, Videm V, Svejgaard A, Svennevig JL, Garred P. Association of mannose-binding-lectin deficiency with severe atherosclerosis.
Lancet. 1998;352:959-960.
[33] Best LG, Davidson M, North KE, MacCluer JW, Zhang Y, Lee ET, Howard BV, DeCroo S, Ferrell RE.Prospective analysis of mannose-binding lectin genotypes and coronary artery disease in American Indians: the Strong Heart Study. Circulation. 2004;109:471-475.
[34] Ohlenschlaeger T, Garred P, Madsen HO, Jacobsen S. Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. N Engl J Med 2004;351:260-267.
[35] Rugonfalvi-Kiss S, Endresz V, Madsen HO, Burian K, Duba J, Prohaszka Z, Karadi I, Romics L, Gonczol E, Fust G, Garred P. Association of Chlamydia pneumoniae with coronary artery disease and its progression is dependent on the modifying effect of mannose-binding lectin. Circulation 2002;106:1071-1076.
[36] Garred P, Madsen HO, Halberg P et al. Mannose-binding lectin polymorphisms and susceptibility to infection in systemic lupus erythematosus. Arthritis Rheum. 1999;42:2145-2152.
[37] Seelen MA, Trouw LA, van der Hoorn JW, Fallaux-van den Houten FC, Huizinga TW, Daha MR, Roos A. Autoantibodies against mannose-binding lectin in systemic lupus erythematosus. Clin Exp Immunol 2003;134:335-43.
[38] Takahashi R, Tsutsumi A, Ohtani K, Goto D, Matsumoto I, Ito S, Wakamiya N, Sumida T. Anti-mannose binding lectin antibodies in sera of Japanese

patients with systemic lupus erythematosus. Clin Exp Immunol 2004;136:585-590.
[39] Mok MY, Jack DL, Lau CS, Fong DY, Turner MW, Isenberg DA, Lydyard PM Antibodies to mannose binding lectin in patients with systemic lupus erythematosus. Lupus 2004;13:522-528.
[40] Tsutsumi A, Sasaki K, Wakamiya N, Ichikawa K, Atsumi T, Ohtani K, Suzuki Y, Koike T, Sumida T. Mannose binding lectin gene: polymorphisms in Japanese patients with systemic lupus erythematosus, rheumatoid arthritis and Sjogren's syndrome. Genes Immun 2001;2:99-104.

FIGURE LEGENDS

Figure 1. Mannose binding lectin protein (MBL) and the MBL gene.

1-A: Four regions comprise the mannose binding lectin (MBL) peptide. The cysteine rich region contains cysteine residues that enable the peptides to form S-S bonds between peptides, and between triple helix components. The collagen domain (tandem repeat of Gly-X-Y sequences) of 3 peptides form a triple helix structure. Carbohydrate recognition domain binds to carbohydrates including mannose and N-acetylglucosamine.

The large red arrow indicate the position of the 3 commonly observed amino acid changes caused by single nucleotide polymorphisms of the MBL gene.

1-B: The mannose binding lectin gene is composed of 4 exons and 3 introns. There are five common single nucleotide polymorphisms (SNP). Each of these SNP has a large effect on the serum concentration of MBL. H/L, X/Y, A/B, A/C, A/D are the commonly used nomenclatures for the alleles of these SNPs. These SNPs combine to make 6 common haplotypes, *HYA*,*LYA*, *LXA*, *HYD*, *LYB* and *LYC*, and strongly influence serum MBL concentration.

1-C: Possible relationship between mannose binding lectin and autoimmunity. The structure of mannose binding lectin (MBL) enables it to discriminate between self and invading microorganisms. MBL would not bind to normal viable cells. However, cells undergoing apoptosis will be recognized by MBL. MBL bound apoptotic cells are engulfed by phagocytes. MBL also binds to DNA. Therefore, MBL has a role in the clearance of potential autoantigens. In addition, MBL binds to various bacteria and viruses, and will aid in host defense through

opsonization or activation of the lectin pathway of the complement cascade. Thus, MBL may aid in protection against some unknown microorganism related to autoimmunity, and is also important when an individual is under immunosupression. It should be noted, that while MBL may be protective against occurrence of autoimmune diseases, it has a role in various tissue injuries, and may pathogenic in certain autoimmune conditions.

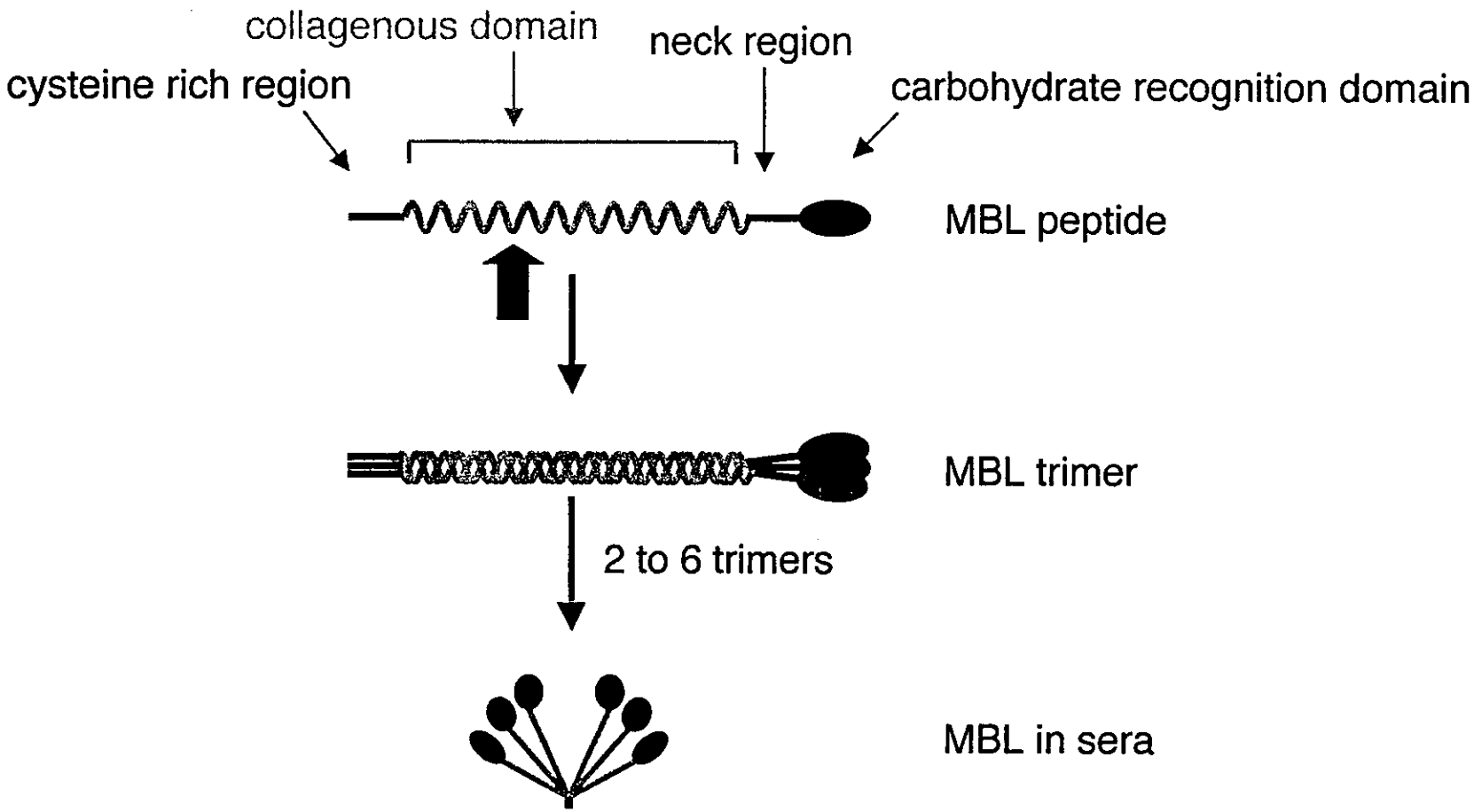
Table. Concentration of mannose binding lectin (MBL) in sera of Japanese individuals with different MBL haplotypes.

Haplotypes	n	MBL concentration (mg/l)	Standard deviation
HYA/LYA	15	1.411	0.548
HYA/HYA	18	1.177	0.481
LYA/LYA	1	1.210	-
HYA/LXA	5	0.830	0.321
LYA/LXA	5	0.584	0.338
HYA/LYB	24	0.333	0.310
LYA/LYB	9	0.119	0.056
LXA/LYB	3	0.013	0.023
LYB/LYB	16	0.002	0.008
total	96	0.622	0.634

See text and figure for definition of haplotypes.

From Tsutsumi et al. [40]

1-A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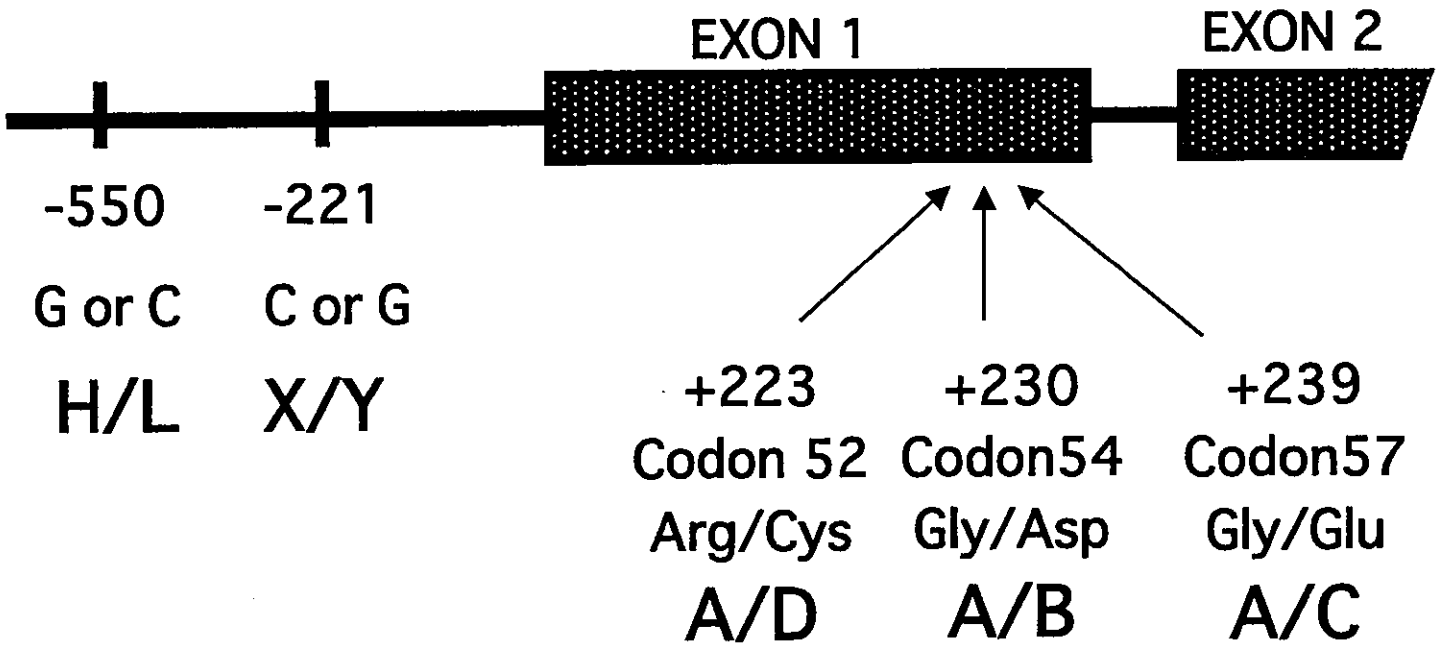
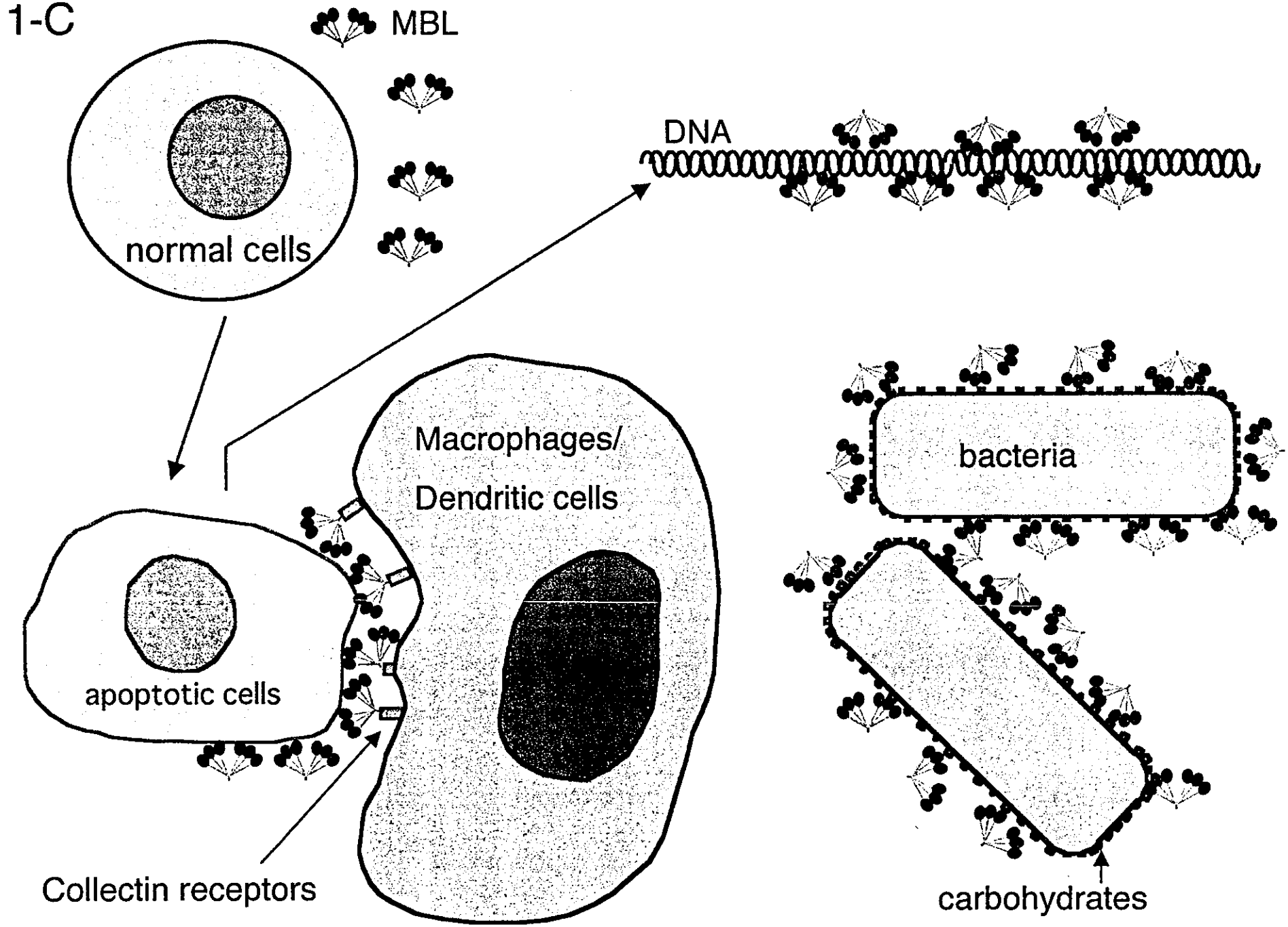
neck region
cysteine rich region
carbohydrate recognition domain
MBL peptide
MBL trimer
2 to 6 trimers
MBL in sera

1-B


EXON 1
EXON 2
-550
-221
G or C
C or G
H/L
X/Y
+223
+230
+239
Codon 52
Codon54
Codon57
Arg/Cys
Gly/Asp
Gly/Glu
A/D
A/B
A/C


1-C
MBL
normal cells
DNA
Macrophages/
Dendritic cells
bacteria
apoptotic cells
Collectin receptors
carbohydrates

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TCRVα14^{+} NKT cells function as effector T cells in collagen-induced arthritis mice

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Summary

Natural killer (NK) T cells are a unique, recently identified cell population and are suggested to act as regulatory cells in autoimmune disorders. In the present study, designed to investigate the role of NKT cells in arthritis development, we attempted to induce arthritis by immunization of type II collagen (CIA) in Jα281 knock out (NKT-KO) and CD1d knock out (CD1d-KO) mice, which are depleted of NKT cells. From the results, the incidence of arthritis (40%) and the arthritis score (1.5 ± 2.2 and 2.0±2.7) were reduced in NKT-KO and CD1d-KO mice compared to those in respective wild type mice (90%, 5.4±3.2 and 2.0±2.7, $P<0.01$). Anti-CII antibody levels in the sera of NKT-KO and CD1d-KO mice were significantly decreased compared to the controls (OD values; 0.32±0.16 and 0.29±0.06 vs. 0.58±0.08 and 0.38±0.08, $P<0.01$). These results suggest that NKT cells play a role as effector T cells in CIA. Although the cell proliferative response and cytokine production in NKT-KO mice after the primary immunization were comparable to those in wild type mice, the ratio of activated T or B cells were lower in NKT-KO mice than wild type mice after secondary immunization (T cells: 9.9±1.8% vs