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Waldenström macroglobulinemia–immunophenotype and natural history

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ABSTRACT

Despite encouraging progress in recent years, our knowledge of the natural history of Waldenström macroglobulinemia (WM), a low–grade LPL (lymphoplasmacytic lymphoma) of mature IgM$^+$ B–lymphocytes, remains superficial. This is particularly true of the etiology of WM (tumor causation and initiation) and the sequence of events that underlie the malignant transformation of precursor B cells (tumor progression). Here we briefly review the epidemiology of and genetic predisposition to WM and consider the role of autoimmunity and chronic inflammation in related tumor development. We discuss the immunophenotypic features of WM, including the immunological specificity of WM–associated IgM paraproteins. The proclivity of patients with WM to develop the rare immunoglobulin autoantibody syndromes mixed IgM–IgG cryoglobulinemia, chronic cold agglutinin disease, and IgM neuropathy will also be discussed. We conclude with a call for additional research to elucidate outstanding questions, such as the role of T cell–dependent *vs*. –independent immune responses in the pathophysiology of WM.

Keywords: Waldenström macroglobulinemia, lymphoplasmacytic lymphoma, mature IgM$^+$ B–lymphocyte, serum paraprotein, genetic predisposition, autoantibody syndrome

INCIDENCE AND AGE OF ONSET

With an annual overall incidence rate of approximately 3 cases per one million persons, Waldenström macroglobulinemia (WM) accounts for a little less than 2% of non–Hodgkin lymphomas in the United States. This number corresponds to approximately 1,500 new cases per year[1]. Incidence in men and Caucasians is higher than in woman and African Americans, respectively[2,3], and the median age at diagnosis is approximately 70 years [4]. Reliable population–based incidence data from the People´s Republic of China are not available at this juncture, but newly reported single–center studies from China are beginning to fill the gap. For example, a retrospective analysis of 93 patients with newly diagnosed WM at Peking Union Medical College Hospital has found that the excess of Chinese men relative to woman (male–to–female ratio: 2.44) [5] is essentially the same as in the US; however, the median age of disease onset in China was 6 years earlier (64 years, range 33–85 years). Another retrospective analysis of 653 patients with B–cell chronic lymphoproliferative disorders from the First Affiliated Hospital of Nanjing Medical University determined that the proportion of WM/LPL (lymphoplasmacytic lymphoma) was higher (5.4%) than one would have been expected in Western countries [6]. These findings are preliminary and await confirmation in larger studies, yet they point to interesting differences in the epidemiology of WM in China and Western countries.

The strongest risk factor for WM is IgM MGUS (monoclonal gammopathy of undetermined significance), the premalignant precursor condition of WM. On average, IgM MGUS progresses to frank WM, or a related disease among the B–cell proliferative disorders, at an annual rate of 1.5% [7,8]. This means that in a hypothetical cohort of 150 individuals with IgM

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MGUS, only one per annum will go onto develop frank WM. Obviously, the slow pace of tumor progression causes difficulty in designing intervention trials that are sufficiently powered to produce clear outcomes in a timely fashion. For example, a controlled clinical trial that tries to answer the question whether a particular drug or chemoprevention method delays the onset of frank WM in individuals with IgM MGUS may take decades to produce a meaningful statistical result. This backdrop provides a strong rationale for developing surrogate endpoints–other than manifestation of frank neoplasia–for new strategies of primary tumor prevention designed to block the MGUS–to–WM transition. Experimental model systems of WM that provide definitive outcomes in reasonably short time frames may lend themselves to that end. Models of this sort will be described in another review article to be published in this journal at a later time.

ETIOLOGY

Just like many other types of cancer, the etiology of WM is unknown. What is abundantly clear, however, is the involvement of two co–factors in disease causation: autoimmunity and chronic inflammation ***(Fig.1)***. The research studies that arrived at this conclusion included hundreds of thousands of individuals from different patient and population cohorts interrogated with a variety of statistical methods. Four investigations shall be briefly summarized in the following: 2 of them were based on surveys performed in the US, and the other 2 were population studies carried out in Sweden. With regard to the US studies, two nationwide surveys of veterans explored the role of antigen stimulation in the pathogenesis of WM. The first survey included 146,394 individuals infected with HCV (hepatitis C virus) and 572,293 controls. It revealed that HCV infection increased the risk of developing WM approximately 3–fold [9]. The second survey identified 361 patients with WM among 4 million veterans, followed for up to 27 years. It found a ~2.5–fold elevated risk of WM in individuals with a personal history of an autoimmune disease and a smaller but still notably elevated risk conferred by hepatitis, rickettsiosis and infection with human immunodeficiency virus (HIV)[10]. The Swedish studies produced similar results. The first one evaluated 2,470 patients with LPL/WM and 9,698 matched controls–together with almost 30,000 first–degree relatives. It uncovered an association of LPL/WM with a personal history of autoimmune diseases (e.g. Sjögren syndrome, hemolytic anemia, polymyalgia rheumatica, and giant cell arteritis) or infectious diseases (e.g. pneumonia, pyelonephritis, sinusitis, herpes zoster, and influenza). Additionally, it revealed a link of LPL/WM with a family history of Sjögren syndrome, autoimmune hemolytic anemia, Guillain–Barré syndrome, cytomegalovirus, gingivitis or periodontitis, and chronic prostatitis[11]. The second study assessed 5,403 patients with MGUS and 21,209 matched controls including the patients´ first–degree relatives. It showed that a personal and family history of autoimmune disease were independently associated with increased risk of MGUS. A personal history of infection and chronic inflammation (but not a family history) was also linked to elevated risk of MGUS[12]. These findings furthered our understanding of the underlying cause of WM by implicating sustained B–cell stimulation with self– and/or exogenous antigens in the pathophysiology of the disease.

A more broadly designed epidemiologic approach recently compared and contrasted the risk factor profiles of WM and other types of non–Hodgkin lymphoma (NHL)[13]. This investigation pooled individual data from 17,471 NHL cases and 23,096 controls from 20 case–control studies from the International

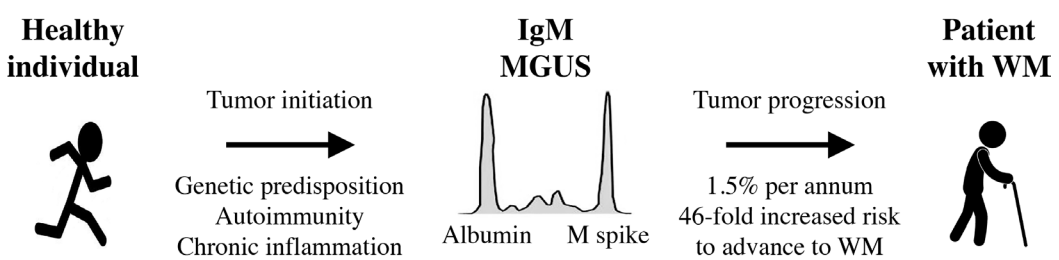

Fig. 1. **Natural history of WM.** No definitive cause of WM has been identified. Autoimmune and inflammatory conditions increase the risk of WM [11], but the underlying biological mechanism remains enigmatic. IgM MGUS is a pre–malignant expansion of a single clone of aberrant lymphoplasmacytic cells that always precedes the emergence of frank WM [13]. IgM MGUS confers, on average, a 46–fold elevation of the risk to progress to WM [7]. Evidence for a genetic predisposition to MGUS is currently emerging [14–16]. Identification of pathways that govern the progression of IgM MGUS to WM may be key for chemoprevention approaches to WM.

Lymphoma Epidemiology Consortium (InterLymph) and estimated associations of NHL subtypes and self–reported medical history, family history of hemato–logical malignancy, lifestyle factors, and occupation. Data analysis showed that risks differed significantly among NHL subtypes for medical history factors (au–toimmune diseases, HCV seropositivity, eczema, and blood transfusion), family history of leukemia and multiple myeloma, alcohol consumption, cigarette smoking, and certain occupations. In contrast, gener–ally homogeneous risks among NHL subtypes were observed for family history of NHL, recreational sun exposure, hay fever, allergy, and socioeconomic sta–tus. Importantly, for WM and 3 other B–cell lympho–mas (marginal zone lymphoma, Burkitt and Burkitt–like lymphoma or leukemia, and diffuse large B–cell lymphoma) increased risks were found for B–cell–activating autoimmune disease and HCV seropositivity. These findings underlined the etiologic heterogeneity among NHL subtypes and led the authors to conclude that some risk factors are common among NHL sub–types, while others are distinct. Thus, WM–specific mechanisms of tumor development that depend on a specific combination of environmental risk factors and genetic susceptibility are likely to exist[13]. The ge–netic susceptibility part of this equation will be con–sidered in the following.

GENETIC PREDISPOSITION

Familial clustering of LPL/WM–which has been repeatedly documented in the past, including stud–ies going back to the 1980s [14–17]–points to inherited traits or "WM alleles" that predispose affected in–dividuals to IgM MGUS and WM[18]. The concept that WM alleles segregate in extended families that exhibit genetic proclivity to WM was underlined by a recent study that involved 1,539 patients with WM and 605 patients with LPL. The result showed that first–degree relatives of patients with LPL/WM had a significantly increased risk of developing one of 3 diseases or conditions: firstly, WM; secondly, MGUS, including IgM, IgG and IgA; thirdly, a type of NHL, such as B–CLL that is related to WM in terms of bi–ology and clinical features[19]. It follows that common B–lineage cancer susceptibility alleles that render carriers susceptible to both LPL/WM and a number of "kindred" lymphoproliferative disorders must ex–ist[20,21]. A dominant or co–dominant genetic trait ap–pears to be more probable for these alleles than a re–cessive trait[22]. A population–based study on familial WM in northern parts of Sweden recently confirmed and extended these findings. Of 12 families, 9 (75%) families and 5 (42%) families, respectively, reported autoimmune diseases and hematological malignancies other than WM in their medical history. Furthermore, an unusually high proportion of abnormal serum pro–tein electrophoresis results were found in 56 relatives of patients with WM: 12 (21%) relatives had MGUS and 13 (23%) relatives had aberrant serum immuno–globulin levels (abnormally low) or patterns (poly– or oligoclonal expansion). These findings lent further support to the hypothesis that the etiology of WM de–pends on both immune–related and genetic factors[23].

An important goal of genetic predisposition re–search concerns the difficult task of identifying the WM alleles postulated above. Projects along this line often begin with genome–wide linkage analysis also known as GWAS (genome wide association stud–ies) of affected individuals *vs*. unaffected controls. This was first accomplished by Mary McMaster and her associates at the US National Cancer Institute, Bethesda, Maryland. Included in her analysis were 11 families at high risk for WM, involving 122 individu–als of which 34 (28%) and 10 (8%) had frank WM and IgM MGUS, respectively. Strong evidence of link–age was found on chromosomes 1q and 4q, but ad–ditional WM loci were suspected on chromosomes 3 and 6[24]. Subsequent work uncovered allelic variants of *IL10* (interleukin 10), *TNFSF10* (tumor necrosis factor superfamily member 10, also known as TRAIL), *IL6* (interleukin 6), and *BCL2* (B cell leukemia 2, apoptosis regulator) as candidate WM genes [25]. The latter two are of particular interest because an incre ase in *BCL2* expression has been implicated in both enhanced B–lymphocyte survival in a variety of conditions and hyper–gamma globulinemia in familial WM [26]. IL–6 not only is up–regulated in LPL–WM cells [27] but also plays an important role in the natural history of multi–ple myeloma (MM) and malignant plasma cell tumors in laboratory mice [28–31]. In this regard, it is interest–ing to note that the genetic susceptibility of WM and MM overlaps[32].

Investigators are now beginning to take advantage of a more recently developed, genome–wide research tool to elucidate genetic susceptibility alleles in WM: whole–exome sequencing (WES). This tool was used to investigate an index fa mily exhibiting coinher–itance for WM. For comparison, 246 independent or sporadic cases of WM were included. Two allelic var–iants of *LAPTM5* (lysosomal protein transmembrane 5) and *HCLS1*(hematopoietic cell–specific Lyn sub–strate) were identified as candidate WM alleles in 3 of 3 patients of the index family. The same variants were found to be present in 8% of unrelated familial cases, 0.5% of non–familial cases, and 0.05% of the con–

trol group. Although these genes are "new" to WM–that is, they have not been implicated thus far in the natural history of the disease–the circumstance that their expression is highly restricted to B cells supports the contention that they play a functional role in WM development[33]. Although the findings above require independent confirmation, it is obvious that WES will be helpful to identify genetic markers that predict progression from IgM MGUS to frank WM [34]. This is a critical knowledge gap in WM. Another priority for future research is the elucidation of gene and environment interactions; i.e., how do genetic polymorphisms cooperate with environmental factors (e.g. infectious agents) to drive WM? Well designed and sufficiently powered population–based studies[35] (in conjunction with genetically engineered animal models of WM in which new insights and hypotheses from human studies can be readily tested and validated) may be key for further progress in our understanding of the genetic network of WM[36].

IMMUNOPHENOTYPE

WM cells exhibit an immunophenotype that is consistent with a mature IgM^+ B–cell poised to enter the pathway of plasmacytic differentiation, but not able to complete it by reaching the end–stage of differentiation: the fully mature, Ig–producing plasma cell ***(Fig.2)***. The molecular mechanism of this curious differentiation block is unclear. The κ to λ light–chain usage of the hallmark mIgM protein is skewed in favor of the former, with a ratio of 5 to 1. In the serum of healthy adults, this ratio is 2 to 1. The reason for the biased utilization of κ light–chains is not known.

The lymphocytoid portion of the tumor cell clone typically expresses surface IgM in conjunction with pan–B cell markers, including CD19, a B cell receptor (BCR) co–receptor that decreases the threshold for antigen–dependent stimulation of BCR signaling; CD20, a phosphoprotein that optimizes humoral immune responses to T–independent (TI) antigens; CD22, a sialic acid–binding, immunoregulatory transmembrane lectin that prevents over activation of the immune system that may result in autoimmunity; CD45, a protein tyrosine phosphatase; and CD79a, an accessory component of the BCR known as Igα. Nuclear immunoreactivity of lymphocytoid cells includes BCL2 (B–cell CLL/lymphoma 2, an anti–apoptotic oncoprotein) and PAX5 (paired box 5 also known as B–cell specific activator protein, which is important for B–cell development and maintenance of lineage fidelity). The cluster of differentiation antigen CD5 (a hallmark of B1 B–cells that mitigates activating signals from the BCR) is variably expressed; a recent study reported 5% to 20% of cases to be positive (usually weakly) for this surface marker. CD10, a membrane metallo–endopeptidase that is also known as neprilysin or common acute lymphoblastic leukemia antigen (CALLA), as well as CD23 and CD103 (*ITGAE*–encoded integrin) are mostly absent.

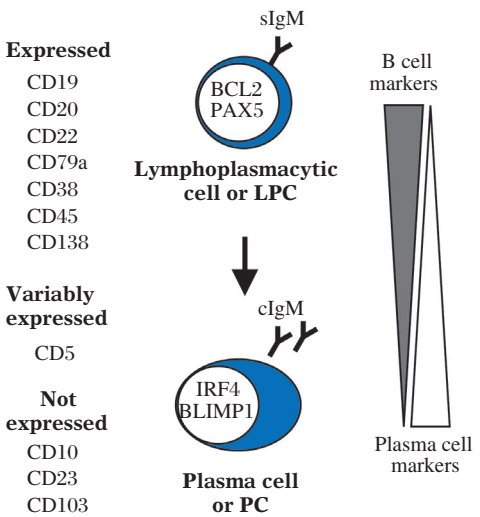

Fig. 2. **Immunophenotype of WM.** Surface markers expressed, variably expressed, or not expressed on WM cells are shown on the left. Nuclear immunoreactivity of lymphocytoid and plasmacytoid cells includes BCL2/PAX5 and IRF4/BLIMP1, respectively. Immunohistochemical analysis of nuclear protein expression of WM cells demonstrated that less mature $CD138^+PAX5^+$ plasma cells were significantly more abundant in WM than in marginal zone lymphoma (MZL) or plasma cell myeloma (also known as multiple myeloma or MM); conversely, more mature $CD138^+IRF4^+$ cells were rare in WM relative to MZL and myeloma [41].

The plasmacytoid portion of the tumor cell clone typically expresses cytoplasmic IgM that is secreted into the serum, in conjunction with B–cell markers; e.g. CD19 and CD45, and plasma cell markers; e.g. CD38 (cyclic ADP ribose hydrolase) and CD138 (syndecan 1). This indicates, as mentioned above, that the cells are arrested at an intermediate plasmablast–like or pre–plasmacyte–like stage of differentiation. Nuclear immunoreactivity includes IRF4 (interferon regulatory factor 4) and PRDM1(PR domain containing 1, with ZNF domain, better known as BLIMP1). A recent immunohistochemical study of nuclear and membrane marker protein expression on WM cells agreed with the flow cytometric pattern described above. Thus, $CD138^+PAX5^+$ plasma cells, which are not fully mature, were found to be more abundant in WM than in marginal zone lymphoma (MZL) or plasma cell

myeloma (also known as multiple myeloma or MM); conversely, mature CD138^{+}IRF4^{+} plasma cells were rare in WM relative to MZL and myeloma [37]. The transcription factor network that governs the peculiar lymphoplasmacytic differentiation arrest of WM cells is poorly understood.

IMMUNOGLOBULIN ANALYSIS

DNA sequence analysis of productively rearranged and expressed Ig variable (IgV) genes has provided important clues on the natural history of CLL (chronic lymphocytic leukemia), mantle cell lymphoma and marginal zone lymphoma–to name but a few neoplasms derived from mature B–lymphocytes for which this approach was successful[38–41]. Inspired by these advances, investigators analyzed the variable portion of IgM heavy– and light–chain genes from both patients with WM and individuals with IgM MGUS. In addition to the skewed κ / λ light–chain ratio mentioned above, research questions included the mutational status of IgV genes (germ line *vs.* mutated *vs.* hyper–mutated), the immune repertoire utilized by WM cell clones (random *vs.* biased usage of V_H or V_L gene families and/or individual genes within these families), the ability of WM cells to perform class switch recombination (CSR; i.e. switching of heavy–chain constant region genes from the "unswitched" or "pre–switch" μ/δ isotypes to the "switched" or "post–switch" γ, α and ε isotypes) and, last but not least, the propensity of WM to undergo tumor heterogeneity[42].

The results of these investigations showed that the great majority of IgM paraprotein V genes harbor somatic mutations, although the number of mutations was low in many cases. Additionally, there was evidence for non–random IgV gene usage; e.g. genes from the V_H3 family were more frequently involved in WM than one would have expected by chance. There was little if any support for ongoing diversification of WM cell clones; in other words, the neoplasm appears to be remarkably stable with regard to Ig expression [43–47]. Although WM cells could be coaxed by some investigators to undergo CSR *in vitro*, this appeared to take place at a very low level [48] and, with the exception of one well–documented example [49], did not seem to occur *in vivo*.

A recent examination of 59 patients with WM and 64 individuals with IgM MGUS (n = 123) confirmed and extended the results of the older studies summarized above. The productively rearranged VDJ_H region of IgM was molecularly cloned in 99 of 123 (80%) cases and then analyzed using DNA sequencing: V_H genes were mutated in 94 of 99 (95%) cases. The median rate of mutation was 6.7% (6.7 base–substitution changes per 100 nucleotides sequenced), with a range from 2.1% to 14.5%. Compared to normal B–cells, genes from subgroup V_H3 were over represented in both WM and IgM MGUS cells, whereas genes from subgroups V_H1 and V_H4 were under represented. The results were interpreted to mean that WM cells have antigen experience [50]. The nature of these antigens shall be described below ***(Fig.3)***.

AUTOIMMUNE AND NATURAL ANTIBODIES

The determination of the immunological specificity of the hallmark monoclonal IgM protein may lead to new insights into the natural history of WM. It may reveal for example "primordial" antigens that stimulate WM precursor B cells at early stages of tumor development, at which the precursor is still dependent on antigen–induced BCR signaling in order to survive. As already mentioned in the first part of this review[51], the clinical presentation of patients with WM, or individuals with IgM MGUS, sometimes provides clues about the immunological specificity of the associated IgM [52]. This is particularly true in cases in which the

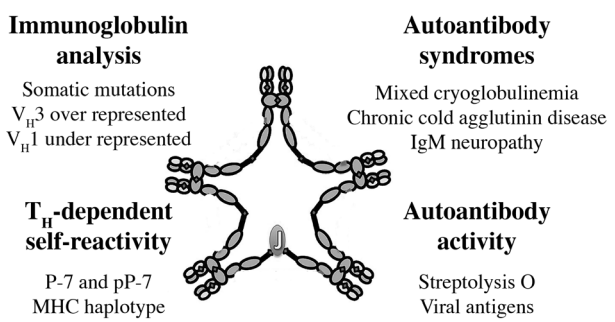

Fig. 3. Origin and pathogenic properties of WM–associated IgM paraproteins. Shown in the center is a scheme of serum IgM, which occurs under normal conditions in pentameric form. Five IgM monomers, each consisting of two μ heavy chains and two κ or μ light chains, are covalently linked by the J or joining chain, resulting in a supramolecular complex that is often depicted as a snow flake or five–leafed shamrock. The pentameric structure of IgM results in a large molecular mass (~970 kilodalton), high avidity to antigen (10 antigen–binding sites), and high potential for complement activation. However, the flip side of these features is poor diffusion properties, low concentration in interstitial fluids, and poor ability to leave the blood stream. In patients with WM, elevated concentrations of monoclonal IgM can lead to serum hyperviscosity, a key distinguishing feature of the disease. IgM may also be deposited in tissues sites, such as kidney, intestine and skin. In some patients, IgM exhibits reactivity to bacterial or viral antigens and leads to autoimmune diseases such as MC, CAD and IgM neuropathy. See text for details.

immunoglobulin is autoreactive and, therefore, functions as an antibody that binds a specific self–antigen and causes autoimmunity. WM has been firmly linked to three autoantibody syndromes, designated mixed IgM–IgG cryoglobulinemia (MC) [53–55], chronic cold agglutinin disease (CAD) [56–57], and IgM neuropathy [58]. These syndromes are increasingly recognized by investigators in China, as evidenced by a recent report on CAD in Chinese patients[59]. Self–antigens associated with these syndromes are the Fc portion of IgG (MC), so–called I/i epitopes on red cells (CAD) and neural carbohydrates (neuropathy). However, it should be added that there is doubt whether these auto– or self–antigens represent the actual ligands that induce IgM signaling in WM precursors in the first place. Some evidence indicates that the original antigens are exogenous in nature, possibly including HCV in cases of MC, bacterial LPS(hpopdysaccharide) in case of CAD, and various bacteria and viruses in the neuropathies [52]. This would be in line with our current understanding of the etiology of WM discussed in a previous section.

The literature on WM paraproteins includes numerous case reports on additional mIgM autoantibody specificities, suggesting that the three autoimmune syndromes mentioned above comprise only the tip of the iceberg. Examples of autoantibodies include reactivity to cytomegalovirus[60] and, as described decades ago by Jan Waldenström himself, streptolysin–O[61]. This provides additional ammunition for implicating exogenous antigens in WM. It is also possible that WM–associated autoantibodies, and perhaps many WM–associated IgM paraproteins for which the immunological specificity has not yet been determined, are in fact natural antibodies [62] produced by natural effector or some other kind of memory B cell. Natural antibodies, which can be readily detected in neonates and young children without prior exposure to pathogen, are immunoglobulins that exhibit a low affinity to antigen and poly–reactivity to a large number of self– and non–self antigens. In conclusion, autoimmune symptoms of patients with WM may be brought about by the cross–reactivity of IgM to exogenous and endogenous antigens.

T CELL–SELECTED IgM PARAPROTEIN

Investigators in Germany have recently come up with an alternative explanation of the genesis of WM–associated paraproteins. They proposed that mIgM is in fact a product of a T–cell–dependent (TD) immune response. Using a relatively new method of Ig analysis called expression cloning [63], the investigator team determined the immunological specificity of IgM paraproteins from patients with WM and individuals with IgM MGUS. They discovered several common self–antigens that were dubbed "paraprotein targets", or "paratargs" for short. This includes a phosphorylated form of paratarg–7, termed pP–7. Compared to non–carriers, carriers of pP–7 were found to have a 6.5–fold higher risk for developing IgM MGUS and WM [64] and an 8– to 13–fold elevation of the risk for IgA/G MGUS and MM[65]. Biochemical studies showed that the phosphorylation of P–7 occurred at serine 17 (S17), a residue that is strategically located in the paraprotein–binding epitope of the self–antigen. Analysis of the CD4 T–cell response to peptides derived from both P–7 and pP–7 in affected and non–affected family members demonstrated that pP–7 induced a strong HLA–DQ– and HLA–DR–restricted immune response. However, P–7 did not. This suggested that pP–7 confers sustained auto–stimulation of cognate helper T–cells, leading, in turn, to the activation of B–cells with high–affinity binding to pP–7. These B–cells appear to undergo clonal expansion until IgM MGUS is manifest, and the progress in some cases to frank WM. Co–immunoprecipitation studies identified PKCζ (protein kinase zeta) and PP2A (protein phosphatase 2A) as the principal kinase and phosphatase at P–7 S17, respectively. This agrees with a new report that the dephosphorylation of pP–7 may be defective in pP–7 carriers due to the inactivation of PP2A[66]. Taken together, the results indicate that only those pP–7–carrying individuals are at elevated risk of developing WM that have both low PP2A activity and the requisite MHC (major histocompatibility complex) class Ⅱ haplotype for the cognate interaction of antigenic pP–7 peptides and $CD4^+$ T–cells. When those preconditions are in place, WM precursors may be generated in a TD immune response. Because the mechanism described above may explain, at least in part, the different prevalence of IgM MGUS/WM in different ethnic backgrounds, it may be worthwhile and productive to evaluate the "pP–7 hypothesis" in the highly diverse ethnic populations in China.

KEY POINTS AND FUTURE DIRECTIONS

The immunophenotype of WM is well established and the autoantibody activity of a subset of WM–associated paraproteins has been elucidated. Still lacking, however, is a detailed analysis of the immunological specificity of IgM paraproteins with respect to underlying "primordial" antigens. This is a difficult area of research, plagued with a host of technical problems that have rendered this field of study unproductive in the past. The future may be brighter, though, because in addition to expression cloning used

in the "paratarg" studies above, new methods are now available that may hold greater promise for identifying antigens and haptens of WM–associated IgM. Epitope–mediated antigen prediction (E–MAP) [67] and high–density peptide microarrays[68] are two interesting developments in this regard. From a natural history point of view, it is conceivable that both endogenous (self) antigens and exogenous (microbial) antigens trigger WM precursors. Shedding light on this issue is of great relevance for the long–standing, thorny issue of the cellular origin of WM[69].

Acknowledgements

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