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Biochemical characterization of monoclonal Immunoglobulin-M in Waldenström Macroglobulinemia

Wouter Verhaar[1*], Nienke Oskam[2,3*], Pleuni Ooijevaar-de Heer[2], Karima Amaador[1], Ninotska I.L. Derksen[2], Marie José Kersten[1], Josephine M.I. Vos[1,2†], Theo Rispens[2,3†]

*,† These authors contributed equally

[1] Department of Hematology, Amsterdam University Medical Centers, University of Amsterdam, Cancer Center Amsterdam, Amsterdam.

[2] Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, Amsterdam.

[3] Amsterdam Institute for Infection and Immunity, Amsterdam, the Netherlands.

Background

Waldenström Macroglobulinemia (WM) is a highly heterogeneous disease and the clinical manifestations correlate poorly with IgM levels. Patients may present with high tumor infiltration and be asymptomatic and vice versa. This strongly suggests that disease manifestations are, at least partially, attributed to variations in biochemical or immunological properties of the monoclonal IgM. In healthy individuals, IgM circulates as a pentameric molecule that consists of five covalently linked monomers (H2L2 pairs), a joining (J-) chain and one CD5-like (CD5L) molecule (**Figure 1A**)[1]. It is known that without the presence of J-chain, IgM predominantly assembles as a hexamer. Previous studies with small sample sizes suggest that monoclonal IgM may be secreted as hexamers, which are potentially more pathogenic than their pentameric counterparts. Assessment of monoclonal IgM composition in larger studies may therefore provide more insight into WM pathogenesis and heterogeneity. Here, we present a pilot study aimed at developing new assays to determine IgM composition and polymerization state in the context of WM.

Methods:

Serum samples were selected from IgM MGUS and WM patients based primarily on varying levels of IgM. Sera from healthy donors (HD) undergoing routine screening at Sanquin were collected as controls. In order to detect integrated J-chain, we raised monoclonal antibodies able to bind to the J-chain in IgM and used these to develop novel enzyme-linked immunosorbent assays (ELISAs) to measure IgM-J-chain. Polymerization state of IgM was

assessed by western blot (native). Patient samples were obtained from the B-cell Biobank at Amsterdam University Medical Centers. All patients signed informed consent.

Results:

In this study, a total of 29 sera of IgM MGUS and WM patients were included with IgM levels ranging from 1.2 to 58.2 g/L. Twenty-eight HD were selected for comparison. To assess integrated J-chain in IgM we developed an ELISA to dissociate CD5L (which can interfere with the detection of J-chain) and used a monoclonal antibody against the J-chain as detection (**Figure 1B**). This shows that for all HD the IgM complex contains a J-chain, whereas in patients some clones appear to have lower J-chain contents compared to total IgM levels. Detection of J-chain by western blot (**Figure 1C**) demonstrates similar results compared to the ELISA. The (partly) devoid J-chain clones lead to differential assembly of IgM into various polymers, next to the normal pentameric variant (**Figure 1D**). The absence of a J-chain has functional implications for IgM. Without J-chain, binding of IgM to polymeric immunoglobulin receptor (pIgR, transports IgM to mucosa) and integration of CD5L is impossible. Indeed, **Figure 1E** shows that the clones (partly) devoid of a J-chain have lower pIgR binding and in addition we found that a large fraction of WM is devoid of CD5L.

Conclusion:

We have developed novel assays to study the structural variation of IgM. We found a wide variation in IgM structure in WM and IgM MGUS patients, while this variation was not seen in healthy donor IgM. Exploring these differences may provide valuable insights in the interplay between IgM structure and disease manifestations in IgM paraproteinemia.

Reference to (the redefining of) IgM composition:

[1] Oskam N, den Boer MA, Lukassen MV, Ooijevaar-de Heer P, Veth TS, van Mierlo G, Lai SH, Derksen NIL, Yin V, Streutker M, Franc V, Šiborová M, Damen MJA, Kos D, Barendregt A, Bondt A, van Goudoever JB, de Haas CJC, Aerts PC, Muts RM, Rooijakkers SHM, Vidarsson G, Rispens T, Heck AJR. CD5L is a canonical component of circulatory IgM. Proc Natl Acad Sci U S A. 2023 Dec 12;120(50):e2311265120. doi: 10.1073/pnas.2311265120. Epub 2023 Dec 6. PMID: 38055740; PMCID: PMC10723121.

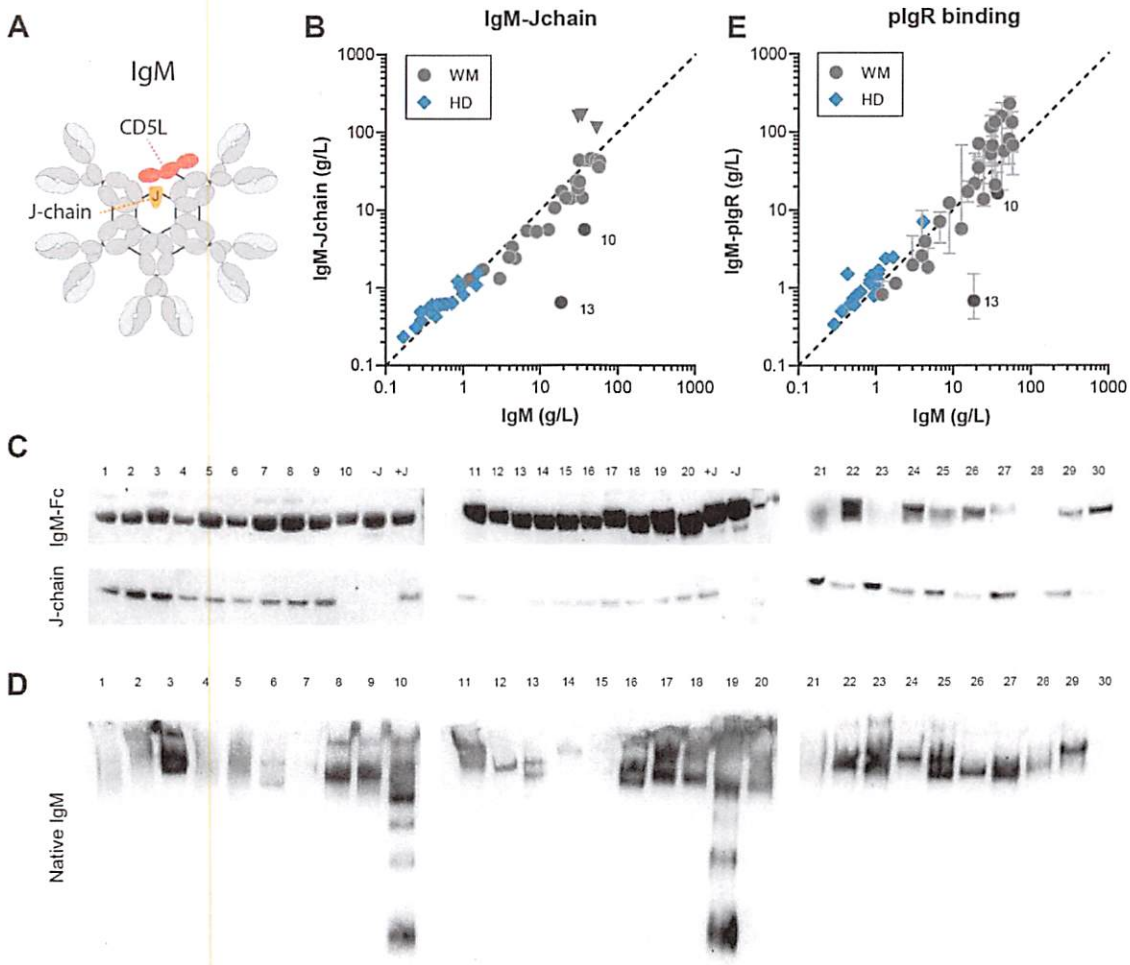

Figure 1. Variable J-chain contents and polymerization of WM monoclonals.

A) Composition of normal serum IgM, consisting of five IgM monomers, a J-chain and one CD5L molecule.

B) Assessment of integrated J-chain in IgM by ELISA with CD5L dissociation and detection with monoclonal anti-J-chain for WM patients (n = 29) and HD (n = 20).

▼*For these three data points, the titration was non-parallel. Consequently, we cannot draw conclusions regarding J-chain content for these samples.*

C) Western blot for IgM- μ chain and J-chain of reduced SDS-PAGE (4-12% Bis-Tris) gel. IgM-Fc at ~75 kDa and J-chain at ~25 kDa.

D) Western blot for IgM-Fc of native PAGE (3-8% Tris-acetate) gel. Bands show natively folded IgM polymers, with the largest polymers at the top of the gel and smaller polymers or monomers running lower. Clone 28 was later identified as IgG-producing LPL, which accounts for the absence of μ-chain and J-chain.

E) Binding of recombinant pIgR (only able to bind antibodies containing J-chain) to IgM was assessed in ELISA for WM patients (n = 29) and healthy donors (n = 20).