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Autophagy, apoptosis, and human leukocyte antigen class II-mediated immune recognition in diffuse large B-cell lymphoma models

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Abstract

This Letter examines evidence that ganoderic acid DM (GA-DM) is associated with caspase activation, changes in autophagy markers, and increased human leukocyte antigen (HLA) class II-restricted interleukin-2 (IL-2) release in a peptide-specific T-cell hybridoma assay using engineered diffuse large B-cell lymphoma (DLBCL) models, and outlines the viability-controlled tissue and ex vivo studies needed to assess autophagic flux and clinical relevance.

Keywords

diffuse large B-cell lymphoma, ganoderic acid DM, human leukocyte antigen class II, antigen presentation, autophagy, apoptosis, CD4+ T-cell recognition

Dear Editor,

We read with interest the study by Blumenstock et al. [1], which examined ganoderic acid DM (GA-DM) at the intersection of regulated cell death and human leukocyte antigen (HLA) class II-restricted immune recognition in diffuse large B-cell lymphoma (DLBCL). In human diffuse large B-cell lymphoma cell line (DB) and Toledo cells, GA-DM reduced viability and induced caspase-dependent cell death [1]. It also increased microtubule-associated protein 1 light chain 3 (LC3) abundance in both its LC3-I and LC3-II forms, the proportion of HLA-DR4-positive cells, and interleukin-2 (IL-2) release by an immunoglobulin G κ-chain peptide spanning residues 188–203 (IgGκ188–203)-specific cluster of differentiation 4-positive (CD4+) T-cell hybridoma [1]. These findings are intriguing, but the observed associations do not by themselves establish a mechanism, particularly in a genetically and biologically heterogeneous disease such as DLBCL [2].

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It remains unclear whether the LC3 response is a protective stress adaptation in surviving lymphoma cells or contributes directly to GA-DM-associated cell death. This distinction matters when considering a therapeutic window, because autophagy can support lymphoma survival or contribute to cell death depending on context [3, 4]. The study assessed LC3 at a single time point without lysosomal turnover assays, and the effect of 3-methyladenine was assessed only in DB cells, where it reduced the IL-2 increase [1]. LC3 accumulation and a single pharmacological inhibitor therefore do not establish increased autophagic flux or a specific causal role for autophagy [5].

Interpretation of the IL-2 data is also complicated by potential differences in viable target-cell number. In separate DB cell experiments, Z-VAD-FMK reduced the loss of viability measured by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay but increased IL-2 release [1]. The higher IL-2 signal may therefore partly reflect a larger number of surviving HLA-DR4-positive target cells rather than more efficient peptide presentation per viable cell. Future assays should normalize IL-2 release to the number of viable lymphoma cells or use viability-matched conditions. Moreover, a therapeutic window cannot be defined without non-malignant B-cell and other immune-cell comparators, together with pharmacologically relevant exposure data.

The HLA-DR4 result also warrants careful interpretation. After 30 μM GA-DM, the proportion of HLA-DR4-positive cells increased from 77.28% to 96.06% in DB cells and from 74.46% to 89.46% in Toledo cells [1]. Because the same exposure substantially reduced MTS-defined viability and the flow-cytometry methods did not report viability-dye exclusion or live-cell gating, selective enrichment of an HLA-DR4-positive surviving fraction cannot be ruled out. This possibility should be distinguished from true upregulation of surface HLA-DR4 on individual viable cells.

The data also do not establish immunogenic cell death, because calreticulin exposure and the release of ATP or high-mobility group box 1 were not assessed [1, 6]. HLA class II-restricted hybridoma activation alone is insufficient to support that conclusion.

Clinical inference is further limited by the experimental design. DB and Toledo cells were engineered to constitutively express HLA-DR4 and were loaded with exogenous IgGκ188–203 peptide before coculture with the peptide-specific 2.18a T-cell hybridoma; IL-2 was the functional readout [1]. This model evaluates exogenous peptide display and hybridoma activation, but it does not establish autophagy-dependent generation of endogenous lymphoma antigens, polyclonal human CD4+ T-cell responses, T-cell priming, cytotoxicity, or in vivo therapeutic efficacy. Autophagy can route intracellular material to HLA class II processing compartments [7]. However, loss of HLA class II expression is well documented in DLBCL [8], and genetic and epigenetic mechanisms underlying HLA class II deficiency have been characterized [9]. Two established cell lines cannot capture the genetic, phenotypic, and microenvironmental heterogeneity of primary disease [2, 9].

Pathology-based validation should integrate morphology and immunophenotype, with molecular annotation where available, to distinguish the neoplastic B-cell compartment from residual non-neoplastic B cells, other immune cells, and stroma. PAX5, CD79a, and CD20 could support B-lineage segmentation, but none is lymphoma-specific in isolation. HLA-DR, cleaved caspase-3, LC3B, p62/SQSTM1, CD4, and CD68 could then be quantified in prespecified tumor and microenvironmental regions. LC3A/B and p62 have also been assessed immunohistochemically in other neoplasms, although such staining should be regarded as a static tissue readout rather than a direct measure of autophagic flux [10]. Multiplex immunohistochemistry or immunofluorescence could assess cell-level associations, whereas digital spatial profiling could characterize selected regions. Relevant clinicopathological variables include cell-of-origin category, MYC/B-cell lymphoma 2 (BCL2) double expression, baseline HLA-DR expression, and treatment context. Archival biopsy cohorts could characterize baseline spatial associations. In fresh primary samples, vehicle- and GA-DM-treated aliquots should be compared under viability-matched conditions, or functional readouts should be normalized to viable target-cell number. Autophagic flux should be assessed by lysosomal blockade and time-resolved LC3-II turnover rather than by static LC3 staining; tandem reporters are better reserved for tractable models [5].

These tissue methods can define localization, density, phenotype, and spatial association, but they cannot demonstrate antigen recognition on their own. Functional validation should therefore include HLA class II blockade together with a viability-controlled ex vivo coculture or another antigen-specific readout. Overall, the study provides a useful starting point. The next step is to determine whether autophagy-related changes, apoptosis, and HLA class II expression are spatially associated with CD4+ T-cell localization in primary DLBCL and whether HLA class II-dependent functional assays confirm antigen recognition.

Abbreviations

CD4+: cluster of differentiation 4-positive

DB: human diffuse large B-cell lymphoma cell line

DLBCL: diffuse large B-cell lymphoma

DR4: human leukocyte antigen DR4 serotype

GA-DM: ganoderic acid DM

HLA: human leukocyte antigen

IgGκ188–203: immunoglobulin G κ-chain peptide spanning residues 188–203

IL-2: interleukin-2

LC3: microtubule-associated protein 1 light chain 3

MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt

Declarations

Author contributions

LP: Conceptualization, Writing—review & editing. VF: Conceptualization, Writing—original draft, Writing—review & editing, Supervision. Both authors read and approved the submitted version.

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The authors declare that they have no conflicts of interest.

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References

1. Blumenstock K, Radwan FFY, Zaman V, Banik NL, Haque A. Crosstalk between autophagy and apoptosis in initiating antitumor immune responses in human lymphoma cells. Explor Immunol. 2025;5:1003216. [DOI] [PubMed] [PMC]
2. Kotlov N, Bagaev A, Revuelta MV, Phillip JM, Cacciapuoti MT, Antysheva Z, et al. Clinical and Biological Subtypes of B-cell Lymphoma Revealed by Microenvironmental Signatures. Cancer Discov. 2021;11: 1468–89. [DOI] [PubMed] [PMC]
3. Levine B, Kroemer G. Biological Functions of Autophagy Genes: A Disease Perspective. Cell. 2019;176: 11–42. [DOI] [PubMed] [PMC]
4. Djavaheri-Mergny M, Giuriato S, Tschan MP, Humbert M. Therapeutic Modulation of Autophagy in Leukaemia and Lymphoma. Cells. 2019;8:103. [DOI] [PubMed] [PMC]
5. Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, Abdellatif M, Abdoli A, Abel S, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition) [1]. Autophagy. 2021;17:1–382. [DOI] [PubMed] [PMC]
6. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. Nat Rev Immunol. 2017;17:97–111. [DOI] [PubMed]
7. Münz C. Autophagy Beyond Intracellular MHC Class II Antigen Presentation. Trends Immunol. 2016; 37:755–63. [DOI] [PubMed]
8. Rimsza LM, Roberts RA, Miller TP, Unger JM, LeBlanc M, Braziel RM, et al. Loss of MHC class II gene and protein expression in diffuse large B-cell lymphoma is related to decreased tumor immunosurveillance and poor patient survival regardless of other prognostic factors: a follow-up study from the Leukemia and Lymphoma Molecular Profiling Project. Blood. 2004;103:4251–8. [DOI] [PubMed]
9. Ennishi D, Takata K, Béguelin W, Duns G, Mottok A, Farinha P, et al. Molecular and Genetic Characterization of MHC Deficiency Identifies EZH2 as Therapeutic Target for Enhancing Immune Recognition. Cancer Discov. 2019;9:546–63. [DOI] [PubMed]
10. Pizzimenti C, Curcio A, Fiorentino V, Germanò A, Martini M, Ieni A, et al. Immunoexpression of autophagy-related proteins in a singlecenter series of sporadic adult conventional clival chordomas. Oncol Lett. 2024;29:32. [DOI] [PubMed] [PMC]