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Loss of B1 and marginal zone B cells during ovarian cancer

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Abstract

Recent advances in immunotherapy have not addressed the challenge presented by ovarian cancer. Although the peritoneum is an "accessible" locus for this disease there has been limited characterization of the immunobiology therein. We investigated the ID8-C57BL/6J ovarian cancer model and found marked depletion of B1 cells from the ascites of the peritoneal cavity. There was also selective loss of the B1 and marginal zone B cell subsets from the spleen. Immunity to antigens that activate these subsets validated their loss rather than relocation. A marked influx of myeloid-derived suppressor cells correlated with B cell subset depletion. These observations are discussed in the context of the housekeeping burden placed on innate B cells during ovarian cancer and to foster consideration of B cell biology in therapeutic strategies to address this challenge.

Keywords

B1 cells; Marginal Zone B cells; Ovarian Cancer; Peritoneal cavity; Tumor microenvironment

1. Introduction

Immunity evolved not only to resist external challenges but also to serve in an internal, "housekeeping" function that operates in the absence of overt inflammation. Apoptotic, senescent, and neoantigen-expressing cells require clearance. In addition, routine, tempered engagement with diverse commensal microflora is essential to ensure epithelial integrity [1]. The immune system is efficient in these capacities for most of our lifespan. However,

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with advancing age homeostasis is challenged by genomic instability, cellular senescence, immune dysregulation, and microbiome changes, factors increasingly shown to foster the emergence of cancer [2].

The barrier role of epithelial linings and the dynamics of their renewal renders their maintenance particularly important [3]. The high mitotic activity of underlying stem cells and proximity to environmental mutagens can promote cancer in epithelial tissue [4]. Typically, cancer evolves over years with the gradual emergence, selection, and persistence of cellular variants that influence their local environment, a hallmark being the temperance of immunity [5]. This slow evolution and cellular complexity confound study of tumor microenvironment (TME) development.

The murine ID8 ovarian cancer (OvCa) model transcends these challenges as a rapidly developing carcinoma in an accessible site rich in immunomodulatory molecules and diverse immunoregulatory cells [6]. Ascites fluid, characterized as an aqueous TME, accumulates in the peritoneal cavity (PerC) as OvCa expands sharing key features found in human serous ovarian carcinoma [7–11]. The peritoneal cavity (PerC) is enriched for B-1 B cells that produce anti-inflammatory IL-10 and “natural” antibodies, primarily IgM, specific for commensal microflora, apoptotic cell ligands, and self-carbohydrates, glycolipids, and glycoproteins. These “innate-like” B cells serve a vital role in housekeeping and controlling inflammation yet have not been investigated in the ID8 model [12].

In this report, changes in B cell biology during OvCa are described. PerC B-1 B cells were found to disappear with advancing disease. Intriguingly, spleen B-1 cells and marginal zone B (MZB) were also depleted, whereas B2 cells persisted. The concomitant loss of a thymus-independent immune responses associated with these B cell subsets confirmed their depletion rather than relocation. This selective loss could reflect the BCR specificity of these subsets and their limited potential for self-renewal [13]. The results are discussed in the context of the stress placed on innate humoral immunity during ovarian cancer.

2. Materials and methods

2.1 Mice

Two- to six-month old female C57BL/6J mice were obtained from the Jackson Laboratory, Bar Harbor, ME. Mice, bred and maintained at Rider University, were handled in accordance with NIH, Animal Welfare Act, and Rider University IACUC guidelines. ID8 (H-2^b)-histocompatible female mice bearing *xid* were generated by crossing H-2^d BALB.*xid* female (X^dX^d) mice with H-2^b C57BL/6J (XY) males to generate H-$2^{b/d}$ F1 male (X^dY) and female (X^dX) mice that were then interbred to generate H-$2^{b/d}$ F2 (X^dX^d, *xid*) and (X^dX, wild-type) females for ID8 transfer. The *xid* trait was tracked by ELISA assessing reduced IgM production [14]. Eight to twenty week old female mice were i.p. injected with 5×10^6 ID8 cells.

2.2 Antigens, immunizations, and ELISA

The commercially available antigens FITC-LPS (TI-1), FITC-Dextran and FITC-Ficoll (TI-2), and FITC-OVA (TD) (Fisher-Thermo, Waltham, MA; Sigma Chemical, St. Louis,

MO) were injected ip. FITC-OVA was emulsified in CFA prior to injection. Sera were collected on day 6 for TI Ags; day 12 for the TD Ag. FITC conjugated to the heterologous carrier BSA (FITC-BSA) was used as the ELISA plating Ag. Absorbance values for pre-immune sera served as the control. ELISA was conducted as described [15].

2.3 In vitro/vivo propagation of ID8 and preparation of cell suspensions

The C57BL/6 ovarian epithelial carcinoma cell line ID8, generously provided by Dr. K.F. Roby, U. Kansas Medical Center, was maintained in DMEM containing 4% FBS, 100 u/ml penicillin, 100 ug/ml streptomycin, 5 ug/ml insulin, 5 ug/ml transferrin, and 5 ng/ml selenite [6]. Cells were washed 2X in sterile PBS prior to injection, 5×10^6 cells in 0.2 mls PBS i.p. Animals were evaluated daily for ascites accumulation and euthanized 5–7 days after abdominal swelling for peritoneal lavage and organ harvest. Mice were euthanized prior to gaining > 20% of their pre-treatment body weight, typically reaching this endpoint 5 to 9 weeks post injection [7,16]. Cell counts and necropsy revealing tumors on the mesothelium validated ID8 expansion

Spleen cell suspensions were generated by gentle mincing with frosted glass slides. RBC depletion was performed with ACK (Ammonium-Chloride-Potassium) lysis buffer followed by washing with Hanks Balanced Salt Solution (HBSS) buffer. PerC cell suspensions were harvested by gentle lavage of the peritoneum. The volume of hemorrhagic ascites was recorded and a fraction subjected to hypotonic lysis to remove RBCs. Viable cell counts were determined by Trypan Blue (Life Technologies) exclusion.

2.4 Immunofluorescence staining and flow cytometry

B lymphocytes and myeloid cells were identified by multiparameter flow cytometric analyses on an Attune NxT (FisherThermo Waltham, MA) or FACSCalibur (Becton Dickinson, Franklin Lakes, NJ) flow cytometer by FSC/SSC gating on lymphocytes and myeloid populations using FlowJo software. 10^6 cells were incubated with a “blocktail” comprised of 2% 2.4G2 MAb (Fc Block, eBioscience), 3% BSA, and 2% normal rat serum (Jackson ImmunoResearch, West Grove, PA) for 20 minutes at 4° C before the addition of fluorochrome-labeled MAbs for an additional 30 minutes on ice. Cells were washed with FACs buffer before analysis.

The following FITC- or Alexa Fluor® 488-,PE-, PerCP-Cy5.5, PE-Cy7-, APC-, Alex Fluor 700, and/or APC-Cy7- labeled MAbs (eBioscience, BioLegend, BD Pharmingen, or R&D Systems) were used: CD11b (M1/70), CD43 (eBioR2/60), CD21/35 (eBio8D9(8D9)), CD23 (B3B4), CD93 (AA4.1), CD19 (eBio1D2(1D3)), CD5 (53–7.3), anti-IgM (eB121-15F9), anti-IgD (11–26c(11–26)), CD45RB (RA3-6B2), anti-Ly6G/Gr-1 (RB6-8C5), anti-Class II MHC (M5/114), anti-F4/80 (BM8) were used.

2.5 Statistical Analyses

Unless specified otherwise, data sets were compared using the Student’s t-test with p-values below 0.05 considered statistically significant. * = P<0.05, ** = P<0.01, *** = P<0.001, **** = P<0.0001compared to each relative control. Multiple testing correction was performed on graphs with multiple t-tests using the Holm-Šídák method.

3. Results

3.1 FACs characterization of hemorrhagic ascites- loss of PerC B-1 cells

Our initial experiments concentrated on characterizing peritoneal cavity (PerC) lymphocyte biology during OvCa development in the ID8/C57BL/6J model [6]. Following ip injection of the ID8 carcinoma cell line, hemorrhagic ascites develops in ~70% of recipients [6,17]. We focused on B cells due to their distinctive subset biology, notably enrichment for B1 cells, in the PerC. Based on both percent representation of all lymphocytes and their total number, multiparameter FACS analyses revealed depletion of $IgM^{+}IgD^{+}$ and $IgM^{++}IgD^{-}$ B cells in mice with ascites. In contrast, $IgM^{+}IgD^{++}$ B cells were increased based on percentage but comparable to control mice based on cell number (Fig. 1A–C). Based on CD5/CD43 expression, the frequency and total number of B1a and B1b cells were reduced in ascites while the frequency of B2 cells increased and their total numbers were unchanged (Fig. 1D–F). These data reveal significant depletion of both B1a and B1b B cells, prominent "housekeeping" cells that are typically enriched in the PerC.

3.2 Spleen B-1 and MZB cells are depleted in mice with OvCa

The loss of peritoneal B-1 cells in hemorrhagic ascites fostered assessing the impact of OvCa on spleen (SP) B cells. As noted in the PerC, $IgM^{+}IgD^{+}$ and $IgM^{++}IgD^{-}$ B cells were depleted in the spleen of mice with OvCa. Again, the percentage of $IgM^{+}IgD^{++}$ B cells increased but the actual number of B2 cells was lower (Fig. 2A–C). Unlike in the PerC, the percentage of SP B1 cells was similar to the control. However, as in the PerC, both B1 subsets, B1a ($CD5^{lo}CD43^{+}$) and B1b ($CD5^{-}CD43^{+}$), were reduced in the spleen (Fig. 2D–F). In addition, another "innate-like" B cell subset associated with "housekeeping", marginal zone B (MZB; $CD21/35^{+}CD1d^{+}$) cells, was also depleted in the SP of mice with OvCa (Fig. 2F–I) [18].

3.3 Transitional B cell depletion during OvCa

In addition to having "housekeeping" functions, B1 cells exhibit limited reconstitution from bone marrow (BM) sourced from adult mice [19,20]. In contrast, B2 cells are replenished from BM throughout adult life [20]. To assess B cell production during OvCa, we monitored SP transitional B cells, the recent BM emigrants defined by correlated CD93/CD19/IgM/CD23 expression [21] (Fig. 3). Although the number of both mature ($CD93^{-}$) and immature ($CD93^{+}$) SP B cells were reduced during OvCa, the percentage of mature cells was unchanged (Fig. 3A–C). The number of each of the three (T1, T2, T3) transitional subsets was reduced during OvCa (Fig. 3E). However, the percentage of the T3 subset, the immediate precursors for B2 ($IgM^{+}IgD^{++}$) cells, was greater than the control (Fig 3A,D). In contrast, the percentage of T2 cells was reduced (Fig. 3A,D). Overall, the transitional B cell data were consistent with the B cell subset alterations observed for mature SP B cells [22].

3.4 MDSC expansion in the PerC of mice with OvCa

A hallmark of cancer progression is immune suppression typically manifest by a diverse array of regulatory white blood cells [23]. PerC cells are enriched for macrophages, which have been shown to suppress lymphocyte activation *in vitro* [24,25]. Assessing the myeloid

fraction of hemorrhagic ascites, we observed a marked influx of myeloid-derived suppressor cells (MDSCs) characterized by expression of CD11b and Gr-1 and lacking markers typically associated with macrophages (Class II MHC, F4/80; Fig. 4A–C). MDSCs were also prominent in the SP, however, unlike in the PerC, a small number of $CD11b^{+}Gr\text{-}1^{+}$ cells were typically found in the SP of control mice (not shown). The presence of MDSCs in the PerC of mice with OvCa reinforces the proposal that hemorrhagic ascites acts as an "aqueous" TME [7].

3.5 Mice bearing xid resist OvCa

In addition to steady state production of "natural" antibodies directed against microflora, B1 cells constitutively produce IL-10 which tempers inflammation and cell-mediated immunity [26]. We speculated that the PerC B1 loss during OvCa might increase inflammation and accelerate disease progression. To test this hypothesis, we assessed OvCa development in mice expressing the X-chromosome-linked immune deficiency, *xid* [27]. These mice have markedly fewer B1 cells due to defective Bruton's tyrosine kinase, *btk*, signaling [28, 29]. Compared with wild-type littermates, mice bearing defective *btk* had slower progression to ascites and more of them had no signs of overt disease (Fig. 5). These results were consistent with prior research showing better anti-cancer immunity when *btk* signaling is inhibited [30–34].

3.6 Loss of TI-2 antigen response in mice with OvCa

The loss of specific B cell subsets from specific anatomic locations did not preclude their relocation to unassessed body sites. To address this concern, mice were immunized with thymus-dependent (TD) and thymus-independent (TI) antigens. B1 and MZB cells are responsible for the type 2 TI (TI-2) antigen response as well as a small portion of the TD response typically associated with B2 cells; all B cells respond to TI-1 antigens, albeit with B1 and MZB cells responsible for a significant portion of this response [18,35]. Mice with OvCa had significantly reduced TI-2 responses compared to controls, while TI-1 and TD responses remained relatively intact (Fig. 6A). Detailed analyses were performed on the TI responses between control mice, those receiving ID8 but not developing ascites, and those with ascites. While responses to the TI-1 antigen were not significantly different between the groups ($p = 0.27$), there were significant differences between groups responding to TI-2 antigens ($p = 0.0016$). Further analysis determined that significantly fewer mice with ascites responded to TI-2 antigens than control mice ($p = 0.0073$; Fig. 6B). These results provide functional evidence for the selective loss of B1 and MZB cells in mice experiencing the hemorrhagic ascites associated with OvCa.

4. Discussion

Nearly all ovarian cancers are epithelial in origin and the majority metastasize into the peritoneum, a site with distinct B cell biology in mice [13,36,37]. However, there has been little research of the impact of OvCa on PerC B cells. Herein, we report that PerC B1 B cells were depleted in mice with hemorrhagic ascites. In contrast, "conventional" (B2) B cells persisted. B1 depletion was also observed in the spleen which also exhibited a reduction of MZB cells. Reduced immunity to antigens that engage these B cell subsets

validated their absence. The loss of B cells correlated with an influx of immunosuppressive myeloid cells, a feature found in human ovarian cancer [38]. These results demonstrate that an evolving cancer can induce the selective loss of B cell subsets, particularly those that maintain tissue homeostasis. They also validate the utility of the ID8 OvCa model to study cancer development via ascites, an accessible, “liquid” TME [39].

The question remains as to why these subsets were particularly sensitive to OvCa. An obvious factor that could account for the loss of B1 cells is their distinctive distribution. Upon activation, B1 cells rapidly accumulate in aggregates of peritoneal adipose tissue, particularly within the milky spots of the omentum and splenoportal fat [40–43]. These fat-associated lymphoid clusters (FALC) are a source of “natural helper” cells that support B1 renewal and IgA production [44]. Typically having a high B1:B2 cell composition, the omentum becomes “clogged” in OvCa patients, with impaired drainage contributing to ascites accumulation [39,45,46]. This proangiogenic, “premetastatic niche” creates a TME enriched with anti-inflammatory cells and adipocytes whose lipids fuel not only tumor growth, but also the distinctive fatty acid metabolism of B1 cells [41,47–50]. The success of omentectomy as a treatment strategy validates the importance of this site in OvCa progression [51–53]. As MZB cells are optimally positioned to filter blood, they are likely engaged in the earliest responses to shed OvCa antigens and they too have recently been found to have similar metabolic requirements, specifically lipid uptake, as B1 cells [50, 54]. Thus, the anatomic distribution of these cells positions them to address the marked antigen load of the ascites generated during OvCa expansion.

The limited diversity BCR repertoire of MZB and B1 cells ensures specificities essential for housekeeping functions [18]. Antibody production directed at expired RBCs, apoptotic/necrotic cells, and commensal microflora are routine functions for these cells [12]. Marked increases in DAMPs, including glycosylated glycoproteins and glycolipids, and lysophosphatidylcholine, are found in the plasma and ascites of ovarian cancer patients, and antibodies directed at these antigens have been shown to control carcinoma in this body site [55–61]. Dying or dead ID8 cells and their extracellular vesicles/exosomes increase antigen burden and tax this housekeeping system [62–64]. The extravascular RBCs and hemoglobin of hemorrhagic ascites are endogenous danger signals [65]. Similar B cell pathology has been observed during the blood-stage of malaria, the stress-induced RBC death (eryptosis) of sepsis, and the RBC-induced apoptotic death of B1 cells during autoimmune hemolytic anemia [63,66–71]. OvCa also fosters dysbiotic inflammation, evidenced as distinct microbiota in the peritoneal fluid, contributing additional antigen burden [72,73]. Linked activation of the BCR and TLR is essential for B-1 activation and costimulatory TLR ligands such as mitDNA (TLR-9), HMGB1 (TLR-2,4,9), histones, LPS (TLR-4), and hsps (TLR-2,4) are found in ascites [74–79]. *In toto*, the volume of DAMPs generated during OvCa may overwhelm the innate, humoral immunity provided by these cells, leading to their “exhaustion” and elimination [80, 81]. The metabolic burden placed on these subsets during their rapid response to these antigens could overwhelm their ability to handle lipid peroxides leading to ferroptosis, an observation recently ascribed specifically to the B1 and MZB subsets, but not B2 cells which have better antioxidant capacity [50]. It is significant to note that the expression of ferroptosis-associated genes is an emerging prognostic biomarker for OvCa [82].

Due to a gene signature associated with plasma cell development, MZB cells have an increased propensity to differentiate into plasma cells [83,84]. Thus, their loss could reflect rapid differentiation into plasmablasts. However, marked immunoglobulin production was not observed during OvCa (not shown). Nonetheless, the loss of these subsets should lead to their replacement by the bone marrow (BM). B1 cells are distinct from B2 cells in their reduced production by BM from older mice [85, 86]. Increased homeostatic proliferation would be required for their self-renewal further increasing their metabolic load and the resultant oxidative stress [87,88]. This could particularly be the case with hematopoiesis disrupted by the inflammation associated with OvCa [89]. A novel form of emergency hematopoiesis, emergency myelopoiesis (EM), has been described for OvCa, where B cell precursors are subverted to produce immunosuppressive, macrophage-like B cells resulting in reduced B1 and increased B2 cell representation in the PerC [90]. In similar research, septic peritonitis promoted IL10 production by macrophage-like B cells and increased EM [91]. The DAMP burden of OvCa increases IL10 production by diverse myeloid cells, as well as B1 and MZB cells, fostering EM and generating immunosuppressive myeloid cells, including granulocytic (Gr-1$^+$) MDSCs [92]. High IL6, IL10, and VEGF levels are ascites hallmarks and factors that promote MDSC production [41,93–95]. MDSC infiltration and IL-1 production are known to alter B cell development [96,97]. MDSC are key drivers of immune suppression in the ID8 OvCa model, their removal reducing cancer expansion [38,98]. Mice bearing *xid (btk*$^{-/-}$) have fewer B1 and MZB cells, thus less IL10 production. This might explain their resistance to ascites development when challenged with ID8 [31]. Furthermore, the absence of *btk* reduces immunosuppressive macrophage and B_{reg} function [30,32]. Overall, *xid* appears to enhance cell-mediated immunity (Th1/CTL function) by fostering an immune environment having fewer immunosuppressive IL10-producing cells [99].

Although the ID8 model affords many parallels to the human disease process, there are several, important limitations. OvCa appears later in life, typically post-menopause [100]. The majority of studies using the ID8 model, including those described herein, work with considerably younger mice that lack any history of pregnancy which has been shown to afford some degree of protection in both mice and women [17, 101]. The median year of OvCa onset is 63 years, equivalent to an 18–24 month old mouse [102]. Another distinction is the rate of disease onset which is much faster in the mouse. Still, this model has key hallmarks of human OvCa particularly that of late-stage disease. It is notable that ip injection of NOD-scid IL2R$\gamma^{-/-}$ (NSG) mice with tumor cell aggregates derived from fresh ovarian biopsy tissues reproduces many features of human OvCa, including hemorrhagic ascites [103].

Reduced numbers of B cells in the ascites of ovarian cancer patients have correlated with negative patient outcomes [104–107]. The loss of MZB and B1 cells described herein fits this pattern. IgA production, a key function of B1 cells, is known to control OvCa as well as provide immunity to commensal microflora thus tempering dysbiosis and its associated cancer-promoting inflammation [108–111]. Gut T cell priming against microflora, a key factor in generating the peripheral memory T cell pool, is curtailed by the loss of these subsets [112,113]. Via IL10 production and PDL1 expression, B1 cells temper inflammation; IL10 ablation promotes tumor growth and increases MDSC generation

[114–116]. Considering the limited success of T cell-focused, checkpoint inhibition therapy for OvCa, strategies that consider B cell biology continue to emerge [110,117,118]. There is growing appreciation for the innate, natural antibody response to cancer neoantigens and the positive outcomes of tumor-infiltrating B and plasma cells [55,56,119–124]. Since it is one of the earliest, most fundamental forms of immunity, we would posit that strategies to enhance the innate humoral response to ovarian cancer are likely to improve patient outcomes.

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Abbreviations:

DAMPs	Damage-associated molecular patterns
FACS	Fluorescein isothiocyanate
FITC	Fluorescence-activated cell sorter
MDSC	myeloid-derived suppressor cell
OvCa	ovarian cancer
PerC	peritoneal cavity
SP	spleen
TD	thymus dependent
TI	thymus independent
TME	tumor microenvironment

References

[1]. Shukla A, Chen C, Jellusova J, Leung CR, Kao E, Bhat N, Lin W, Apgar JR, Rickert RC. Self-reactive B cells in the GALT are actively curtailed to prevent gut inflammation, JCI Insight 4(16) (2019) e130621. https://pubmed.ncbi.nlm.nih.gov/31335327/

[2]. Hanahan D, Hallmarks of cancer: new dimensions, Cancer Discovery 12(1) (2022) 31–46. https://pubmed.ncbi.nlm.nih.gov/35022204/ [PubMed: 35022204]

[3]. Brock CK, Wallin ST, Ruiz OE, Samms KM, Mandal A, Sumner EA, Eisenhoffer GT, Stem cell proliferation is induced by apoptotic bodies from dying cells during epithial tissue maintenance, Nature Communications 10(1) (2019) 1044–1055. 10.1038/s41467-019-09010-6

[4]. Tomasetti C, Li L, Vogelstein B, Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention, Science 355 (2017) 1330–1334. https://pubmed.ncbi.nlm.nih.gov/28336671/ [PubMed: 28336671]

[5]. Schreiber RD, Old LJ, Smyth MJ, Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion, Science 331 (2011) 1565–1570. https://pubmed.ncbi.nlm.nih.gov/21436444/ [PubMed: 21436444]

[6]. Roby KF, Taylor CC, Sweetwood JP, Cheng Y, Pace JL, Tawfik O, Persons DL, Smith PG, Terranova PF, Development of a syngeneic mouse model for events related to ovarian cancer, Carcinogenesis, 21(4) (2000) 585–591. https://pubmed.ncbi.nlm.nih.gov/10753190/ [PubMed: 10753190]

[7]. Rodriguez GM, Galpin KJC, McCloskey CW, Vanderhyden BC, The tumor microenvironment of epithelial ovarian cancer and its influence on response to immunotherapy, Cancers (Basel) 10(8) (2108) 242–250. https://pubmed.ncbi.nlm.nih.gov/30042343/

[8]. Leinster DA, Kulbe H, Everitt G, Thompson R, Perretti M, Gavins FNE, Cooper D, Gould D, Ennis DP, Lockley M, McNeish IA, Nourshargh S, Balkwill FR, The peritoneal tumour microenvironment of high-grade serous ovarian cancer, J. Pathol 227(2) (2012) 136–145. https://pubmed.ncbi.nlm.nih.gov/22322968/ [PubMed: 22322968]

[9]. Li Y, Zhou J, Zhuo Q, Zhang J, Xie J, Han S, Zhao S, Malignant ascite-derived extracellular vesicles inhibit T cell activity by upregulating Siglec-10 expression, Cancer Manag Res 11 (2019) 7123–7134. https://pubmed.ncbi.nlm.nih.gov/31534365/ [PubMed: 31534365]

[10]. Worzfeld T, von Strandmann EP, Huber M, Adhikary T, Wagner U, Reinartz S, Muller R, The unique molecular and cellular microenvironment of ovarian cancer, Front Oncol 7 (2017) 24–35. https://pubmed.ncbi.nlm.nih.gov/28275576/ [PubMed: 28275576]

[11]. Wilson AJ, Barham W, Saskowski J, Tikhomirov O, Chen L, Lee H-J, Yull F, Khabele D, Tracking NF-kB activity in tumor cells during ovarian cancer progression in a syngeneic mouse model, J Ovarian Res 6(1) (2013) 63–72. https://pubmed.ncbi.nlm.nih.gov/24020521/ [PubMed: 24020521]

[12]. van Kaer L, Postoak JL, Song W, Wu L, Innate and innate-like effector lymphocytes in health and disease, J Immunol 209(2) (2022) 199–207. https://pubmed.ncbi.nlm.nih.gov/35821102/ [PubMed: 35821102]

[13]. Smith FL, Baumgarth N, B-1 cell responses to infections, Curr Opin Immunol 57 (2019) 23–31. https://pubmed.ncbi.nlm.nih.gov/30685692/ [PubMed: 30685692]

[14]. CBA/CaHN-*Btk*^{xid}/J, Strain #: 001011. https://www.jax.org/strain/001011

[15]. Pennello A, Taylor J, Matlack R, Karp J, Riggs J, Spiny mice (*Acomys cahirinus*) do not respond to thymus-independent type 2 antigens, Dev Comp Immunol 30(12) (2006) 1181–1190. https://pubmed.ncbi.nlm.nih.gov/16698082/ [PubMed: 16698082]

[16]. Peterson NC, Behavioral, clinical, and physiologic analysis of mice used for ascites monoclonal antibody production, Comp Med 50(5) (2000) 516–526. https://pubmed.ncbi.nlm.nih.gov/11099135/ [PubMed: 11099135]

[17]. Cohen CA, Shea AA, Heffron CL, Schmelz EM, Roberts PC, The parity-associated microenvironmental niche in the omental fat band is refractory to ovarian cancer metastasis, Cancer Prev Res (Phila) 6(11) (2013) 1182–1193. https://pubmed.ncbi.nlm.nih.gov/24022590/ [PubMed: 24022590]

[18]. Martin F, Oliver AM, Kearney JF, Marginal zone and B1 B cells unite in the early response against T-independent blood-borne antigens, Immunity 14(5) (2001) 617–629. https://pubmed.ncbi.nlm.nih.gov/11371363/ [PubMed: 11371363]

[19]. Rosser EC, Mauri C, Regulatory B cells: origin, phenotype, and function, Immunity 42(4) (2015) 607–612. https://pubmed.ncbi.nlm.nih.gov/25902480/ [PubMed: 25902480]

[20]. Wang Y, Liu J, Burrows PD, J-Y. Wang, B cell development and maturation, Adv Exp Med Biol 1254 (2020) 1–22. https://pubmed.ncbi.nlm.nih.gov/32323265/ [PubMed: 32323265]

[21]. Allman D, Lindsley RC, Demuth W, Rudd K, Shinton SA, Hardy RR, Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation, J Immunol 167(12) (2001) 6834–6840. https://pubmed.ncbi.nlm.nih.gov/11739500/ [PubMed: 11739500]

[22]. Nemazee D, Natural history of MZ B cells, J Exp Med 218(4) (2021) e20202700. https://pubmed.ncbi.nlm.nih.gov/33555296/ [PubMed: 33555296]

[23]. van Vlerken-Ysla L, Tyurina YY, Kagan VE, Gabrilovich DI, Functional states of myeloid cells in cancer, Cancer Cell 41(3) (2023) 490–504. https://pubmed.ncbi.nlm.nih.gov/36868224/ [PubMed: 36868224]

[24]. Goldman N, Valiuskyte K, Londregan J, Swider A, Somerville J, Riggs J, Macrophage regulation of B cell proliferation, Cell Immunol 314 (2017) 54–62. https://pubmed.ncbi.nlm.nih.gov/28238361/ [PubMed: 28238361]

[25]. Matlack R, Yeh K, Rosini L, Gonzalez D, Taylor J, Silberman D, Pennello A, Riggs J, Peritoneal macrophages suppress T-cell activation by amino acid catabolism, Immunology 117 (2006) 386–395. https://pubmed.ncbi.nlm.nih.gov/16476058/ [PubMed: 16476058]

[26]. Bouaziz J-D, Yanaba K, Tedder TF, Regulatory B cells as inhibitors of immune responses and inflammation, Immunol Rev 224 (2008) 201–214. https://pubmed.ncbi.nlm.nih.gov/18759928/ [PubMed: 18759928]

[27]. Wicker LS, Scher I, X-linked immune deficiency (xid) of CBA/N mice, Curr Top Microbiol Immunol 124 (1986) 87–101. https://pubmed.ncbi.nlm.nih.gov/3486747/ [PubMed: 3486747]

[28]. Conley ME, Parolini O, Rohrer J, Campana D, X-linked agammaglobulinemia: new approaches to old questions based on the identification of the defective gene, Immunol Rev 138 (1994) 5–21. https://pubmed.ncbi.nlm.nih.gov/8070817/ [PubMed: 8070817]

[29]. Contreras CM, Halcomb KE, Randle L, Hinman RM, Gutierrez T, Clarke SH, Satterthwaite AB, Btk regulates multiple stages in the development and survival of B-1 cells, Mol Immunol 44(10) (2007) 2719–2728. https://pubmed.ncbi.nlm.nih.gov/17207856/ [PubMed: 17207856]

[30]. Gunderson AJ, Kaneda MM, Tsujikawa T, Nguyen AV, Affara NI, Ruffell B, Gorjestani S, Liudahl SM, Truitt M, Olson P, Kim G, Hanahan D, Tempero MA, Sheppard B, Irving B, Chang BY, Varner JA, Coussens LM, Bruton-tyrosine kinase-dependent immune cell cross-talk drives pancreas cancer, Cancer Discov, 6(3) (2016) 270–285 https://pubmed.ncbi.nlm.nih.gov/26715645/ [PubMed: 26715645]

[31]. Stiff A, Trikha P, Wesolowski R, Kendra K, Hsu V, Uppati S, McMichael E, Duggan M, Campbell A, Keller K, Landi I, Zhong Y, Dubovsky J, Harrison Howard J, Yu L, Harrington B, Old M, Reiff S, Mace T, Tridandapani S, Muthusamy N, Caligiuri MA, Byrd JC, Carlson WE 3rd, Myeloid-derived suppressor cells express Bruton's tyrosine kinase and can be depleted in tumor-bearing hosts by ibrutinib treatment, Cancer Res 76(8) (2016) 2125–2136. https://pubmed.ncbi.nlm.nih.gov/26880800/ [PubMed: 26880800]

[32]. Schwartz M, Zhang Y, Rosenblatt JD, B cell regulation of the anti-tumor response and role in carcinogenesis, J Immunother 4 Cancer (2016) 40–48. https://pubmed.ncbi.nlm.nih.gov/27437104/

[33]. Mangla A, Khare A, Vineeth V, Panday NN, Mukhopadhyay A, Ravindran B, Bal V, George A, Rath S, Pleiotropic consequences of Bruton tyrosine kinase deficiency in myeloid lineages lead to poor inflammatory responses, Blood 104(4) (2004) 1191–1197. https://pubmed.ncbi.nlm.nih.gov/15117762/ [PubMed: 15117762]

[34]. Khare A, Viswanathan B, Gund R, Jain N, Ravindran B, George A, Rath S, Bal V, Role of Bruton's tyrosine kinase in macrophage apoptosis, Apoptosis 16(4) (2011) 334–346. https://pubmed.ncbi.nlm.nih.gov/21193961/ [PubMed: 21193961]

[35]. Mond JJ, Mongini PK, Sieckmann D, Paul WE, Role of T lymphocytes in the response to TNP-AECM-Ficoll, 125(3) (1980) J Immunol 1066–1070. [PubMed: 6774013]

[36]. Hayakawa K, Hardy RR, Herzenberg LA, The "Ly-1 B" cell subpopulation in normal immunodefective, and autoimmune mice. J Exp Med 157(1) (1983) 202–218. https://pubmed.ncbi.nlm.nih.gov/6600267/ [PubMed: 6600267]

[37]. Yousefi M, Dehghani S, Nosrati R, Ghanei M, Salmaninejad A, Rajaie S, Haszanzadeh M, Pasdar A, Current insights into the metastasis of epithelial ovarian cancer- hopes and hurdles, Cell Oncol (Dordr) 43(4) (2020) 515–538. https://pubmed.ncbi.nlm.nih.gov/32418122/ [PubMed: 32418122]

[38]. Baert T, Vankerckhoven A, Riva M, Van Hoylandt A, Thirion G, Holger G, Mathivet T, Vergote I, Coosemans A, Myeloid derived suppressor cells: key drivers of immunosuppression in ovarian cancer, Front Immunol 10 (2019) 1273. https://pubmed.ncbi.nlm.nih.gov/31214202/ [PubMed: 31214202]

[39]. Ford CE, Werner B, Hacker NF, Warton K, The untapped potential of ascites in ovarian cancer research and treatment. Br J Cancer 123(1) (2020) 9–16. [PubMed: 32382112]

[40]. Clark R, Krishnan V, Schoof M, Rodriguez I, Theriault B, Chekmareva M, Rinker-Schaeffer C, Milky spots promote ovarian cancer metastatic colonization of peritoneal adipose in

experimental models, Am J Pathol 183(2) (2013) 576–591. https://pubmed.ncbi.nlm.nih.gov/23885715/ [PubMed: 23885715]

[41]. Liu M, Silva-Sanchez A, Randall TD, Meza-Perez S, Specialized immune responses in the peritoneal cavity and omentum, J Leukoc Biol 109(4) (2021) 717–729. https://pubmed.ncbi.nlm.nih.gov/32881077/ [PubMed: 32881077]

[42]. Ha S, Tsuji M, Suzuki K, Meek B, Yasuda N, Kaisho T, Fagarasan S, Regulation of B1 cell migration by signals through Toll-like receptors, J Exp Med, 203(11) (2006) 2541–2550. https://pubmed.ncbi.nlm.nih.gov/17060475/ [PubMed: 17060475]

[43]. Gerber SA, Rybalko VY, Bigelow CE, Lugade AA, Foster TH, Frelinger JG, Lord EM, Preferential attachment of peritoneal tumor metastases to omental immune aggregates and possible role of a unique vascular microenvironment, Am J Pathol 169(5) (2006) 1739–1752. https://pubmed.ncbi.nlm.nih.gov/17071597/ [PubMed: 17071597]

[44]. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J-I, Ohtani M, Fujii H, Koyasu S, Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells, Nature 463(7280) (2010) 540–544. https://pubmed.ncbi.nlm.nih.gov/20023630/ [PubMed: 20023630]

[45]. Ansel KM, Harris RBS, Cyster JG, CXCL13 is required for B1 cell homing, natural antibody production, and body cavity immunity, Immunity, 16(1) (2002) 67–76. https://pubmed.ncbi.nlm.nih.gov/11825566/ [PubMed: 11825566]

[46]. Sangisetty SL, Miner TJ, Malignant ascites: a review of prognostic factors, pathophysiology and therapeutic measures, World J Gastrointest Surg, 4(4) (2012) 87–95. https://pubmed.ncbi.nlm.nih.gov/22590662/ [PubMed: 22590662]

[47]. Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt MR, Romero IL, Carey MS, Mills GB, Hotamisligil GS, Yamada SD, Peter ME, Gwin K, Lengyel E, Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth, Nat Med 17(11) (2011) 1498–1503. https://pubmed.ncbi.nlm.nih.gov/22037646/ [PubMed: 22037646]

[48]. Liu M, Starenki D, Scharer CD, Silva-Sanchez A, Molina PA, Pollock JS, Cooper SJ, Arend RC, Rosenberg AF, Randall TD, Meza-Perez S, Circulating Tregs accumulate in omental tumors and acquire adipose-resident features, Cancer Immunol Res 10(5) (2022) 641–655. https://pubmed.ncbi.nlm.nih.gov/35263766/ [PubMed: 35263766]

[49]. Clarke AJ, Riffelmacher T, Braas D, Cornall RJ, Simon AK, B1a B cells require autophagy for metabolic homeostasis and self-renewal, J Exp Med 215(2) (2018) 399–413. https://pubmed.ncbi.nlm.nih.gov/29326381/ [PubMed: 29326381]

[50]. Muri J, Thut H, Bornkamm GW, Kopf M, B1 and marginal zone B cells but not follicular B2 require Gpx4 to prevent lipid peroxidation and ferroptosis, Cell Rep 29(9) (2019) 2731–2744. https://pubmed.ncbi.nlm.nih.gov/31775041/ [PubMed: 31775041]

[51]. Chen SS, Spiegel G, Stage I endometrial carcinoma. Role of omental biopsy and omentectomy, J Reprod Med 36(9) 627–629. https://pubmed.ncbi.nlm.nih.gov/1774722/

[52]. Oosterling SJ, van der Bij GJ, Bogels M, van der Sijp JRM, Beelen RHJ, Meijer S, van Egmond M, Insufficient ability of omental milky spots to prevent peritoneal tumor outgrowth supports omentectomy in minimal residual disease, Cancer Immunol Immunother 55(9) (2006) 1043–1051. https://pubmed.ncbi.nlm.nih.gov/16311732/ [PubMed: 16311732]

[53]. Etzerodt A, Moulin M, Doktor TK, Delfini M, Mossadegh-Keller N, Bajenoff M, Sieweke MH, Kragh Moestrup S, Auphan-Anezin N, Lawrence T, Tissue-resident macrophage in omentum promote metastatic spread of ovarian cancer, J Exp Med 217(4) (2020) e20191869. https://pubmed.ncbi.nlm.nih.gov/31951251/ [PubMed: 31951251]

[54]. Kinker GS, Vitiello GAF, Ferreira WAS, Chaves AS, de Lima VCC, da Silva Medina T, B cell orchestration of anti-tumor immune responses: a matter of cell localization and communication, Front Cell Dev Biol 9 (2021) 678127. https://pubmed.ncbi.nlm.nih.gov/34164398/ [PubMed: 34164398]

[55]. Haro MA, Dyevoich AM, Phipps JP, Haas KM, Activation of B-1 cells promotes tumor cell killing in the peritoneal cavity, Cancer Res 79(1) (2019) 159–170. https://pubmed.ncbi.nlm.nih.gov/30224373/ [PubMed: 30224373]

[56]. Dyevoich AM, Disher NS, Haro MA, Haas KM, A TLR4-TRIF-dependent signaling pathway is required for protective natural tumor-reactive IgM production by B1 cells, Cancer Immunol Immunother 69(10) (2020) 2113–2124. https://pubmed.ncbi.nlm.nih.gov/32448982/ [PubMed: 32448982]

[57]. Fang X, Gaudette D, Furui T, Mao M, Estrella V, Eder A, Pustilnik T, Sasagawa T, Lapushin R, Yu S, Jaffe RB, Wiener JR, Erickson JR, Mills GB, Lysophospholipid growth factors in the initiation, progression, metastases, and management of ovarian cancer, Ann N Y Acad Sci 905 (2000) 188–208. https://pubmed.ncbi.nlm.nih.gov/10818454/ [PubMed: 10818454]

[58]. Bunt SK, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S, Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression, J Immunol 176(1) (2006) 284–290. https://pubmed.ncbi.nlm.nih.gov/16365420/ [PubMed: 16365420]

[59]. Miyamoto S, Renee Ruhaak L, Stroble C, Salemi MR, Phinney B, Lebrilla CB, Leiserowitz GS, Glycoproteomic analysis of malignant ovarian cancer ascites fluid identifies unusual glycopeptides, J Proteome Res 15(9) (2016) 3358–3376. https://pubmed.ncbi.nlm.nih.gov/27500424/ [PubMed: 27500424]

[60]. Dorsett BH, Ioachim HL, Stolbach L, Walker J, Barber HR, Isolation of tumor-specific antibodies from effusions of ovarian carcinomas, Int J Cancer 16(5) (1975) 779–786. https://pubmed.ncbi.nlm.nih.gov/1184243/ [PubMed: 1184243]

[61]. Disaia PJ, Nalick RH, Townsend DE, Antibody cytotoxicity studies in ovarian and cervical malignancies, Obstet Gynecol 42(5) (1973) 644–650. https://pubmed.ncbi.nlm.nih.gov/4796001/ [PubMed: 4796001]

[62]. Qian Y, Wang H, Clarke SH, Impaired clearance of apoptotic cells induces the activation of autoreactive anti-Sm marginal zone and B-1 B cells, J Immunol 172(1) (2004) 625–635. https://pubmed.ncbi.nlm.nih.gov/14688375/ [PubMed: 14688375]

[63]. Murakami M, Tsubata T, Okamoto M, Shimizu A, Kumagai S, Imura H, Honjo T, Antigen-induced apoptotic death of Ly-1 B cells responsible for autoimmune disease in transgenic mice, Nature 357(6373) (1992) 77–80. https://pubmed.ncbi.nlm.nih.gov/1574128/ [PubMed: 1574128]

[64]. Gong X, Chi H, Franziska Strohmer D, Tobias Teichmann A, Xia Z, Wang Q, Exosomes: a potential tool for immunotherapy of ovarian cancer, Front Immunol 13 (2023) 1089410. https://pubmed.ncbi.nlm.nih.gov/36741380/ [PubMed: 36741380]

[65]. Yin T, He S, Liu X, Jiang W, Ye T, Lin Z, Sang Y, Su C, Wan Y, Shen G, Ma X, Yu M, Guo F, Liu Y, Li L, Hu Q, Wang Y, Wei Y, Extravascular red blood cells and hemoglobin promote tumor growth and therapeutic resistance as endogenous danger signals, J Immunol (2015) 194(1) 429–437. https://pubmed.ncbi.nlm.nih.gov/25429070/ [PubMed: 25429070]

[66]. Achtman A, Khan M, MacLennan ICM, Langhorne J, *Plasmodium chabaudi* infection in mice induces strong B cell responses and striking but temporary changes in splenic cell distribution, J Immunol 171(1) (2003) 317–324. https://pubmed.ncbi.nlm.nih.gov/12817013/ [PubMed: 12817013]

[67]. Bockstal V, Geurts N, Magez S, Acute disruption of bone marrow lymphopoiesis and apoptosis of transitional and marginal zone B cells in the spleen following a blood-stage *Plasmodium chabaudi* infection in mice, J Parasitol Res 534697 (2011) Epub 2011 May 4. https://pubmed.ncbi.nlm.nih.gov/21687602/

[68]. Yoder BJ, Goodrum KJ, *Plasmodium chabaudi chabaudi*: B-1 cell expansion correlates with semiresistance in BALB/cJ mice, Exp Parasitol 98(2) (2001) 71–82. https://pubmed.ncbi.nlm.nih.gov/11465990/ [PubMed: 11465990]

[69]. Lang E, Lang F, Mechanisms and pathophysiological significance of eryptosis, the suicidal erythrocytic death, Semin Cell Dev Biol 39 (2015) 35–42. [PubMed: 25636585]

[70]. Foller M, Huber SM, Lang F, Erythrocyte programmed cell death, IUBMB Life 60(10) (2008) 661–668. https://pubmed.ncbi.nlm.nih.gov/18720418/ [PubMed: 18720418]

[71]. Aziz M, Holodick NE, Rothstein TL, Wang P, B-1a cells protect mice from sepsis: critical role of CREB, J Immunol 199(2) (2017) 750–760. https://pubmed.ncbi.nlm.nih.gov/28630091/ [PubMed: 28630091]

[72]. Miao R, Badger TC, Groesch K, Diaz-Sylvester PL, Wilson T, Ghareeb A, Anne Martin J, Cregger M, Welge M, Bushell C, Auvil L, Zhu R, Brard L, Braundmeier-Fleming A, Assessment

of peritoneal microbial features and tumor marker levels as potential diagnostic tools for ovarian cancer, PLoS One 15(1) (2020) e0227707. https://pubmed.ncbi.nlm.nih.gov/31917801/ [PubMed: 31917801]

[73]. Mahoney DE, Chalise P, Rahman F, Pierce JD, Influences of gastrointestinal microbiota dysbiosis on serum proinflammatory markers in epithelial ovarian cancer development and progression, Cancers (Basel) 14(12) (2022) 3022. https://pubmed.ncbi.nlm.nih.gov/35740687/ [PubMed: 35740687]

[74]. Sm Kreuk L, Koch MA, Slayden LC, Lind NA, Chu S, Savage HP, Kantor AB, Baumgarth N, Barton GM, B cell receptor and Toll-like receptor signaling coordinate to control distinct B-1 responses to both self and the microbiota, Elife 8 (2019) e47015. https://pubmed.ncbi.nlm.nih.gov/31433298/ [PubMed: 31433298]

[75]. Singel KL, Grzankowski KS, Khan A.N.M. Nazmul H., Grimm MJ, D'Auria AC, Morrell K, Eng KH, Hylander B, Mayor PC, Emmons TR, Lenart N, Fekete R, Kornyei Z, Muthukrishnan U, Gilthorpe JD, Urban CF, Itagaki K, Hauser CJ, Leifer C, Moyisch KB, Odunsi K, Denes A, Segal BH, Mitochondrial DNA in the tumour microenvironment activates neutrophils and is associated with worse outcomes in patients with advanced epithelial ovarian cancer, Br J Cancer 120(2) (2019) 207–217. https://pubmed.ncbi.nlm.nih.gov/30518816/ [PubMed: 30518816]

[76]. Jiang C, Qu X, Ke H, Gong W, Chen R, Yang W, Cheng Z, Association between the HMGB1/TLR4 signaling pathway and the clinicopathological features of ovarian cancer, Mol Med Rep 18(3) (2018) 3093–3098. https://pubmed.ncbi.nlm.nih.gov/30015957/ [PubMed: 30015957]

[77]. Zhou M, McFarland-Mancini MM, Funk HM, Husseinzadeh N, Mounajjed T, Drew AF, Toll-like receptor expression in normal ovary and ovarian tumors, Cancer Immunol Immunothera 58(9) (2009) 1375–1385. https://pubmed.ncbi.nlm.nih.gov/19184006/

[78]. Zhang Z, Zhao H, Chu C, Fu X, Liu Y, Wang L, Wei R, Xu K, Li L, Li X, The emerging roles of TLR and cGAS signaling in tumorigenesis and progression of ovarian cancer, Front Pharmacol 13 (2022) 1072670. https://pubmed.ncbi.nlm.nih.gov/36588690/ [PubMed: 36588690]

[79]. Sipos A, Ujlaki G, Maka E, Szabo J, Uray K, Krasznai Z, Bai P, The role of the microbiome in ovarian cancer: mechanistic insights into oncobiosis and to bacterial metabolite signaling, Mol Med 27(1) (2021) 33. https://pubmed.ncbi.nlm.nih.gov/33794773/ [PubMed: 33794773]

[80]. Weiss G, Crompton PD, Li S, Walsh LA, Moir S, Traore B, Kayentao K, Ongoiba A, Doumbo OK, Pierce SK, Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area, J Immunol 183(3) (2009) 2176–2182. https://pubmed.ncbi.nlm.nih.gov/19592645/ [PubMed: 19592645]

[81]. Roe K, NK-cell exhaustion, B-cell exhaustion, and T-cell exhaustion- the differences and the similarities, Immunology 166(2) (2022) 155–168. https://pubmed.ncbi.nlm.nih.gov/35266556/ [PubMed: 35266556]

[82]. Peng J, Hao Y, Rao B, Zhang Z, A ferroptosis-related lncRNA signature predicts prognosis in ovarian cancer patients, Transl Cancer Res, 10(11) (2021) 4802–4816. https://pubmed.ncbi.nlm.nih.gov/35116333/ [PubMed: 35116333]

[83]. Oliver AM, Martin F, Gartland GL, Carter RH, Kearney JF, Marginal zone B cells exhibit unique activation, proliferative and immunoglobulin secretory reponses, Eur J Immunol 27(9) (1997) 2366–2374. https://pubmed.ncbi.nlm.nih.gov/9341782 [PubMed: 9341782]

[84]. Gunn KE, Brewer JW, Evidence that marginal zone B cells possess an enhanced secretory apparatus and exhibit superior secretory activity, J Immunol 17 (6) (2006) 3791–3798. https://pubmed.ncbi.nlm.nih.gov/16951340/

[85]. Baumgarth N, A Hard(y) look at B-1 cell development and function, J Immunol 199(10) (2017) 3387–3394. https://pubmed.ncbi.nlm.nih.gov/29109178/ [PubMed: 29109178]

[86]. Birjandi SZ, Ippolito JA, Ramadorai AK, Witte PL, Alterations in marginal zone macrophages and marginal zone B cells in old mice, J Immunol 186(6) (2011) 3441–3451. https://pubmed.ncbi.nlm.nih.gov/21307289/ [PubMed: 21307289]

[87]. Akkaya M, Pierce SK, From zero to sixty and back to zero again: the metabolic life of B cells, Curr Opin Immunol 57 (2019) 1–7. https://pubmed.ncbi.nlm.nih.gov/30312894/ [PubMed: 30312894]

[88]. Jellusova J, Cross-talk between signal transduction and metabolism in B cells, Immunol Lett 201 (2018) 1–13. https://pubmed.ncbi.nlm.nih.gov/30439477/ [PubMed: 30439477]

[89]. Plackoska V, Shaban D, Nijnik A, Hematologic dysfunction in cancer: mechanisms, effects on antitumor immunity, and roles in disease progression, 13 Front Immunol (2022) 1041010. https://pubmed.ncbi.nlm.nih.gov/36561751/ [PubMed: 36561751]

[90]. Chen C, Park B, Ragonnaud E, Bodogai M, Wang X, Zong L, Lee J-M, Beerman I, Biragyn A, Cancer co-opts differentiation of B-cell precursors into macrophage-like cells, Nat Commun 13(1) (2022) 5376. https://pubmed.ncbi.nlm.nih.gov/36104343/ [PubMed: 36104343]

[91]. Kanayama M, Izumi Y, Akiyama M, Hayashi T, Atarashi K, Roers A, Sato T, Ohteki T, Myeloid-like B cells boost emergency myelopoiesis through IL-10 production during infection, J Exp Med, 220(4) (2023) e20221221. https://pubmed.ncbi.nlm.nih.gov/36719648/ [PubMed: 36719648]

[92]. Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S, Rodriguez PC, Sica A, Umansky V, Vonderheide RH, Gabrilovich DI, Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards, Nat Commun 7 (2016) 12150. https://pubmed.ncbi.nlm.nih.gov/27381735/ [PubMed: 27381735]

[93]. Moughon DL, He H, Schokrpur S, Karen Jiang Z, Yaqoob M, David J, Lin C, Luisa Iruela-Arispe M, Dorigo O, Wu L, Macrophage blockade using CSF1R inhibitors reverses the vascular leakage underlying malignant ascites in late-stage epithelial ovarian cancer, Cancer Res 75(22) (2015) 4742–4752. https://pubmed.ncbi.nlm.nih.gov/26471360/ [PubMed: 26471360]

[94]. Wu L, Deng Z, Peng Y, Han L, Liu J, Wang L, Li B, Zhao J, Jiao S, Wei H, Ascites-derived IL-6 and IL-10 synergistically expand $CD14^{+}HLA\text{-}DR^{-/low}$ myeloid-derived suppressor cells in ovarian cancer patients, Oncotarget 8(44) (2017) 76843–76856. https://pubmed.ncbi.nlm.nih.gov/29100353/ [PubMed: 29100353]

[95]. Horikawa N, Abiko K, Matsuma N, Hamanishi J, Baba T, Yamaguchi K, Yoshioka Y, Koshiyama M, Konishi I, Expression of vascular endothelial growth factor in ovarian cancer inhibits tumor immunity through the accumulation of myeloid-derived suppressor cells, Clin Cancer Res 23(2) (2017) 587–599. https://pubmed.ncbi.nlm.nih.gov/27401249/ [PubMed: 27401249]

[96]. Wang Y, Schafer CC, Hough KP, Tousif S, Duncan SR, Kearney JF, Ponnazhagan S, Hsu H-C, Deshane JS, Myeloid-derived suppressor cells impair B cell responses in lung cancer through IL-7 and STAT5, J Immunol 201(1) 278–295. https://pubmed.ncbi.nlm.nih.gov/29752311/

[97]. Kennedy DE, Knight KL, Inhibition of B lymphopoiesis by adipocytes and IL-1-producing myeloid-derived suppressor cells, J Immunol 195(6) (2015) 2666–2674. https://pubmed.ncbi.nlm.nih.gov/26268654/ [PubMed: 26268654]

[98]. Pekarek LA, Starr BA, Toledano AY, Schreiber H, Inhibition of tumor growth by elimination of granulocytes, J Exp Med 181(1) (1995) 435–440. https://pubmed.ncbi.nlm.nih.gov/7807024/ [PubMed: 7807024]

[99]. Composto G, Gonzalez D, Bucknum A, Silberman D, Gonzalez D, Taylor J, Kozlowski M, Bloomfield T, Bartlett T, Riggs J, Peritoneal T lymphocyte regulation by macrophages, Immunobiology 215 (2010) 256–264.

[100]. Lheureux S, Gourley C, Vergote I, Oza A, Epithelial ovarian cancer, Lancet 393(10177) (2019) 1240–1253. https://pubmed.ncbi.nlm.nih.gov/30910306/ [PubMed: 30910306]

[101]. Adami HO, Hsieh CC, Lambe M, Trichopoulos D, Leon D, Persson I, Ekbom A, Janson PO, Parity, age at first childbirth, and risk of ovarian cancer, Lancet 344(8932) (1994) 1250–1254. https://pubmed.ncbi.nlm.nih.gov/7967985/ [PubMed: 7967985]

[102]. Biomarkers of Aging. Of Mice and Men: Translating Mouse Age to Human Age, https://www.gowinglife.com/of-mice-and-men-translating-mouse-age-to-human-age/, posted on 22 September 2021, (accessed 23 October 2023)

[103]. Bankert RB, Balu-Iyer SV, Odunsi K, Shultz LD, Kelleher RJ, Barnas JL, Simpson-Abelson M, Parsons R, Yokota SJ, Humanized mouse model of ovarian cancer recapitulates patient solid tumor progression, ascites formation, and metastasis, PLoS One 6(9) (2011) e24420. https://pubmed.ncbi.nlm.nih.gov/21935406/ [PubMed: 21935406]

[104]. Lacki J, Markowska J, Wiktorowicz K, Phenotype of lymphocyte in ovarian tumor peritoneal fluid. A preliminary study, Eur J Gynaecol Oncol 13(6) (1992) 475–479. https://pubmed.ncbi.nlm.nih.gov/1473526/ [PubMed: 1473526]

[105]. Berek JS, Bast RC Jr., Lichtenstein A, Hacker NF, Spina CA, Lagasse LD, Knapp RC, Zighelbom J, Lymphocyte cytotoxicity in the peritoneal cavity and blood of patients with ovarian cancer, Obste Gynecol 64(5) (1984) 708–714. https://pubmed.ncbi.nlm.nih.gov/6493663/

[106]. Reijnhart RM, Bieber MM, Teng NN, FACS analysis of peritoneal lymphocytes in ovarian cancer and control patients, Immunobiology 191(1) (1994) 1–8. https://pubmed.ncbi.nlm.nih.gov/7806256/ [PubMed: 7806256]

[107]. Mandell GL, Fisher RI, Bostick F, Young RC, Ovarian cancer: a solid tumor with evidence of normal cellular immune function but abnormal B cell function, Am J Med 66(4) (1979) 621–624. https://pubmed.ncbi.nlm.nih.gov/312015/ [PubMed: 312015]

[108]. Kroese FG, Butcher EC, Stall AM, Lalor PA, Adams S, Herzenberg LA, Many of the IgA producing plasma cells in murine gut are derived from self-replenishing precursors in the peritoneal cavity, Int Immunol 1(1) (1989) 75–84. https://pubmed.ncbi.nlm.nih.gov/2487677/ [PubMed: 2487677]

[109]. MacPherson AJ, Gatto D, Sainsbury E, Harriman GR, Hengartner H, Zinkernagel RM, A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria, Science 288(5474) (2000) 2222–2226. https://pubmed.ncbi.nlm.nih.gov/10864873/ [PubMed: 10864873]

[110]. Biswas S, Mandal G, Payne KK, Anadon CM, Gatenbee CD, Chaurio RA, Costich TL, Moran C, Harro CM, Rigolizzo KE, Mine JA, Trillo-Tinoco J, Sasamoto N, Terry KL, Marchion D, Buras A, Wenham RW, Yu X, Townsend MK, Tworoger SS, Rodriguez PC, Anderson AR, Conenjo-Garcia JR, IgA transcytosis and antigen recognition govern ovarian cancer immunity, 591(7850) Nature (2021) 464–470. https://pubmed.ncbi.nlm.nih.gov/33536615/ [PubMed: 33536615]

[111]. Zorea J, Motro Y, Mazor RD, Koren Carmi Y, Shulman Z, TRAF3 suppression encourages B cell recruitment and prolongs survival of microbiome-intact mice with ovarian cancer, J Exp Clin Cancer Res, 42(1) (2023) 107. https://pubmed.ncbi.nlm.nih.gov/37121997/ [PubMed: 37121997]

[112]. Margry B, Wieland WH, van Kooten PJ, van Eden W, Broere F, Peritoneal cavity B-1a cells promote peripheral CD4+ T-cell activation, Eur J Immunol 43(9) (2013) 2317–2326. https://pubmed.ncbi.nlm.nih.gov/23719868/ [PubMed: 23719868]

[113]. Hand TW, Dos Santos LM, Bouladoux N, Molloy MJ, Pagan AJ, Pepper M, Maynard CL, Elson CO 3rd, Belkaid Y, Acute gastrointestinal infection induces long-lived microbiota-specific T cell responses, Science 337(6101) (2012) 1553–1556. https://pubmed.ncbi.nlm.nih.gov/22923434/ [PubMed: 22923434]

[114]. Khan AR, Hams E, Floudas A, Sparwasser T, Weaver CT, Fallon PG, PD-L1hi B cells are critical regulators of humoral immunity, Nat Commun 6 (2015) 5997. https://pubmed.ncbi.nlm.nih.gov/25609381/ [PubMed: 25609381]

[115]. Chaurio RA, Anadon CM, Costich TL, Payne KK, Biswas S, Harro CM, Moran C, Ortiz AC, Cortina C, Rigolizzo KE, Sprenger KB, Mine JA, Innamarato P, Mandal G, Powers JJ, Martin A, Wang Z, Mehta S, Perez BA, Li R, Robinson J, Kroeger JL, Curiel TJ, Yu X, Rodriguez PC, Conejo-Garcia JR, TGF-β-mediated silencing of genomic organizer SATB1 promotes Tfh differentiation and formation of intra-tumoral tertiary lymphoid structures, Immunity 55(1) (2022) 115–128. https://pubmed.ncbi.nlm.nih.gov/35021053/ [PubMed: 35021053]

[116]. Tanaka T, Moira Wilke C, Kryczek I, Chen GY, Kao J, Nunez G, Zou W, Interleukin-10 ablation promotes tumor development, growth, and metastasis, Cancer Res 72(2) (2012) 420–429. https://pubmed.ncbi.nlm.nih.gov/22123924/ [PubMed: 22123924]

[117]. Ruffin AT, Cillo AR, Tabib T, Liu A, Onkar S, Kunning SR, Lampenfeld C, Atiya HI, Abecassis I, Kurten CHL, Qi Z, Soose R, Duvvuri U, Kim S, Oesterrich S, Lafyatis R, Coffman LG, Ferris RL, Vignali DAA, Bruno TC, B cell signatures and tertiary lymphoid structures contribute to outcome in head and neck squamous cell carcinoma, Nat Commun 12(1) (2021) 3349. https://pubmed.ncbi.nlm.nih.gov/34099645/ [PubMed: 34099645]

[118]. Mazor RD, Nathan N, Gilboa A, Stoler-Barak L, Moss L, Solomonov I, Hanuna A, Divinsky y., Shmueli MD, Hezroni H, Zaretsky I, Mor M, Golani O, Sabah G, Jakobson-Setton A, Yanichkin

N, Feinmesser M, Tsoref D, Salman L, Yeoshoua E, Peretz E, Erlich I, Mendelson Cohen N, Gershoni JM, Freund N, Merbl Y, Yaari G, Eitan R, Sagi I, Shulman Z, Tumor-reactive antibodies evolve from non-binding and autoreactive precursors, Cell, 185(7) (2022) 1208–1222. https://pubmed.ncbi.nlm.nih.gov/35305314/ [PubMed: 35305314]

[119]. Rawat K, Soucy SM, Kolling FW, Manohar Diaz K, King WT, Tewari A, Jakubzick CV, Natural antibodies alert the adaptive immune system of the presence of transformed cells in early tumorigenesis, J Immunol 209(7) (2022) 1252–1259. https://pubmed.ncbi.nlm.nih.gov/36028292/ [PubMed: 36028292]

[120]. Laumont CM, Banville AC, Gilardi M, Hollern DP, Nelson BH, Tumour-infiltrating B cells: immunological mechanisms, clinical impact and therapeutic opportunities, Nat Rev Cancer 22(7) (2022) 414–430. https://pubmed.ncbi.nlm.nih.gov/35393541/ [PubMed: 35393541]

[121]. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, Yizhak K, Sade-Feldman M, Blando J, Han G, Gopalakrishnan V, Xi Y, Zhao H, Amaria RN, Tawbi HA, Cogdill AP, Liu W, LeBleu VS, Kugeraski FG, Patel S, Davies MA, Hwu P, Lee JE, Gershenwalk JE, Lucci A, Arora R, Woodman S, Keung EZ, Gaudreau P-O, Reuben A, Spencer CN, Burton EM, Haydu LE, Lazar AJ, Zapassodi R, Hudgens CW, Ledesman DA, Ong S, Bailey M, Warren S, Rao D, Krijgsman O, Rozeman EA, Peeper D, Blank CU, Schumacher TN, Butterfield LH, Zelazowska MA, McBride KM, Kalluri R, Allison J, Petitprez F, Herman Fridman W, Sautes-Fridman C, Hacohen N, Rezvani K, Sharma P, Tetzlaff MT, Wang L, Wargo JA, B cells and tertiary lymphoid structures promote immunotherapy response, Nature 577 (7791) (2020) 549–555. https://pubmed.ncbi.nlm.nih.gov/31942075/ [PubMed: 31942075]

[122]. Monfort A, Pearce O, Maniati E, Vincent BG, Bixby L, Bohm S, Dowe T, Wilkes EH, Chakravarty P, Thompson R, Topping J, Cutillas PR, Lockley M, Serody JS, Capasso M, Balkwill FR, A strong B-cell response is part of the immune landscape in human high-grade serous ovarian metastases, Clin Cancer Res 23(1) (2017) 250–262. https://pubmed.ncbi.nlm.nih.gov/27354470/ [PubMed: 27354470]

[123]. Kroeger DR, Milne K, Nelson BH, Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer, Clin Cancer Res 22(12) (2016) 3005–3015. https://pubmed.ncbi.nlm.nih.gov/26763251/ [PubMed: 26763251]

[124]. Yang C, Lee H, Jove V, Deng J, Zhang W, Liu X, Forman S, Dellinger TH, Wakabayashi M, Yu H, Pal S, Prognostic significance of B-cells and pSTAT3 in patients with ovarian cancer, PLoS One 8(1) (2013) e54029. https://pubmed.ncbi.nlm.nih.gov/23326565/ [PubMed: 23326565]

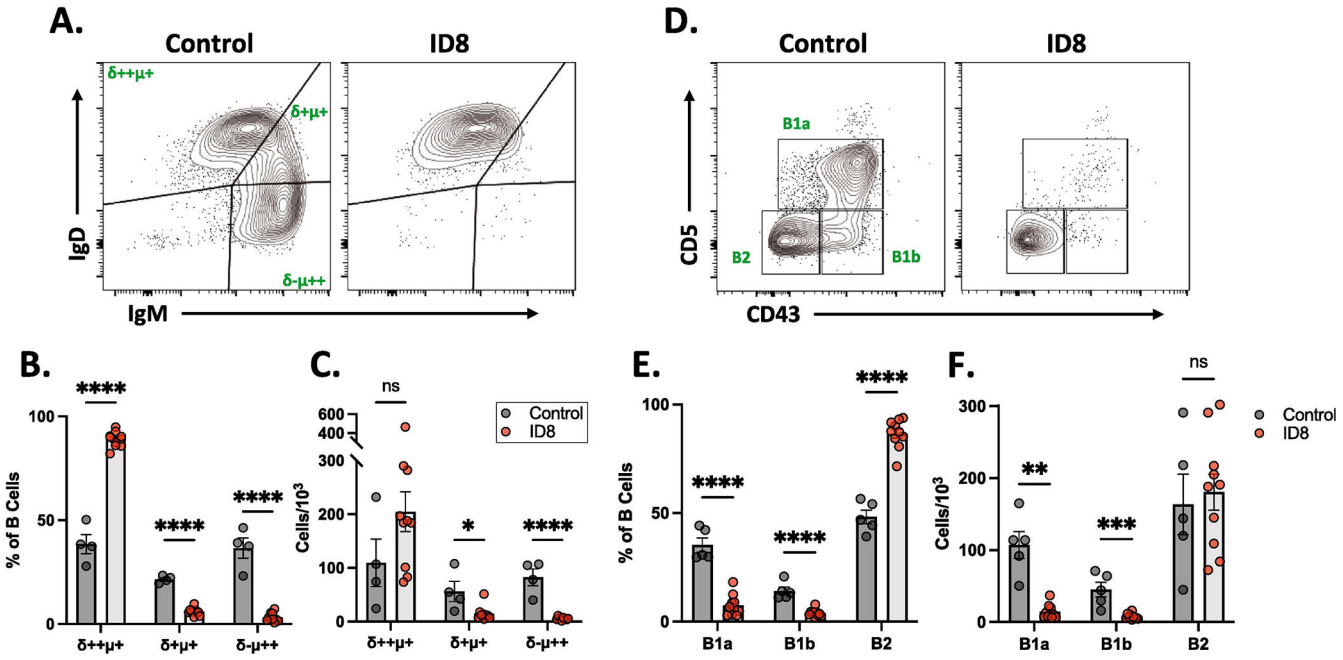

Fig. 1.

PerC B1 B cell subsets are decreased during ovarian cancer. (**A**) Representative flow cytometric profiles of IgM and IgD distribution among PerC B cells. (**B**) Frequency of B cells and (**C**) cells per thousand total cells. δ−, δ+, or δ++ represent IgD-negative, IgD -low, or IgD-high cells; μ+ or μ++ represent IgM-low or IgM-high cells (**D**) Representative profiles of B1 subsets based on CD5 and CD43 expression. (**E**) Frequency of B1 cell subsets and (**F**) cells per thousand total cells. FACS profiles were pre-gated on single, lymphocytes, CD19+. Data sets compared using unpaired t tests ***p<0.05 ****p<0.01 *****p<0.001 ******p<0.0001

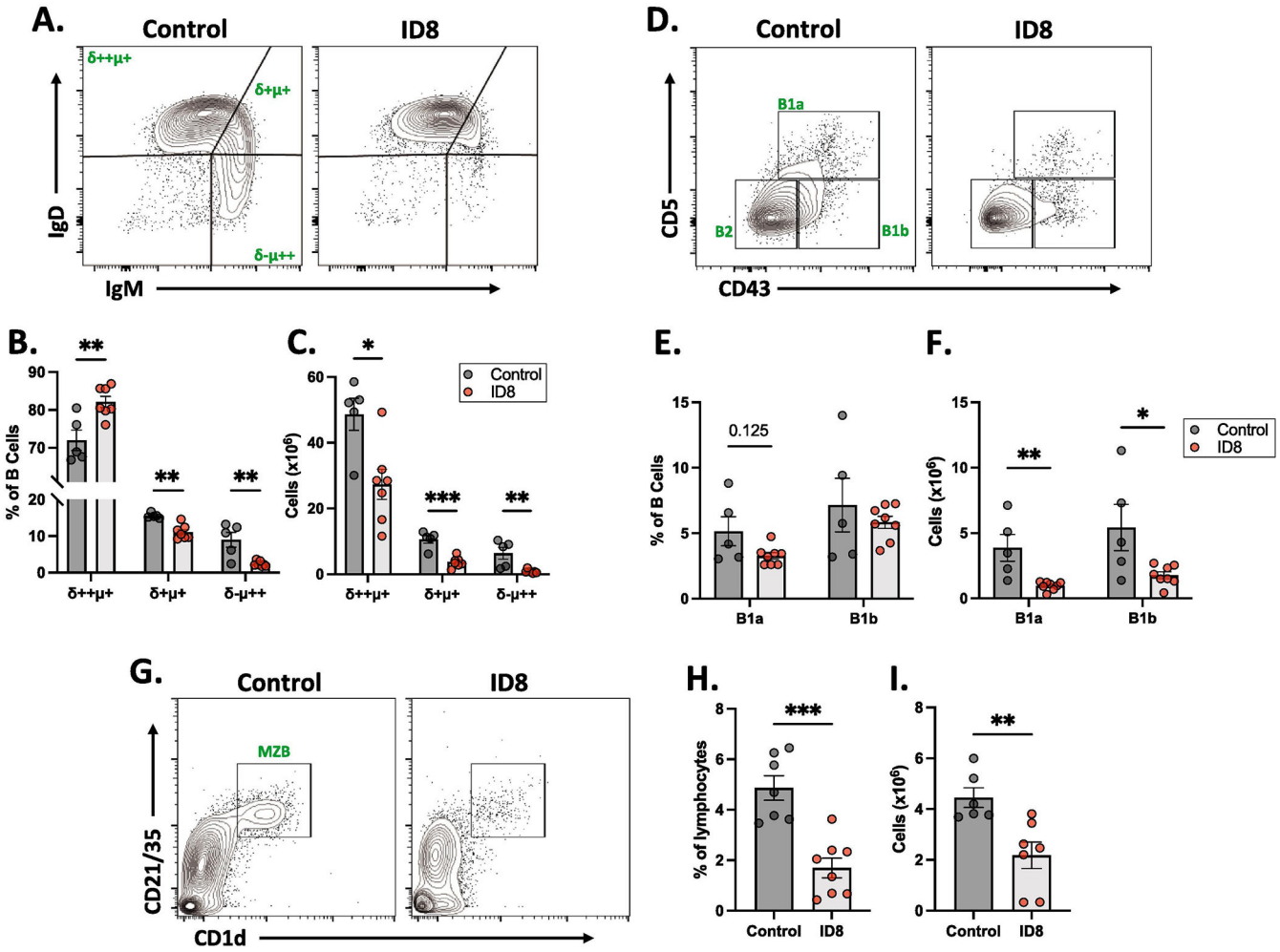

Fig. 2.

Spleen B1 and MZB cells are decreased during ovarian cancer. (**A**) Representative flow cytometric profiles of IgM and IgD distribution among spleen B cells. (**B**) Frequency of B cells and (**C**) total numbers. (**D**) Representative profiles of spleen B1 subsets based on CD5 and CD43 expression. (**E**) Frequency of B cell subsets and (**F**) total numbers. (**G**) Representative profiles of MZB cells based on CD1d and CD21/35 expression. (**H**) Frequency of MZB B cells and (**I**) total numbers. (**A-F)** FACS profiles were pre-gated on single, lymphocytes, CD19+. (**G-I)** FACS profiles were pre-gated on single, lymphocytes. Data sets compared using unpaired t tests *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

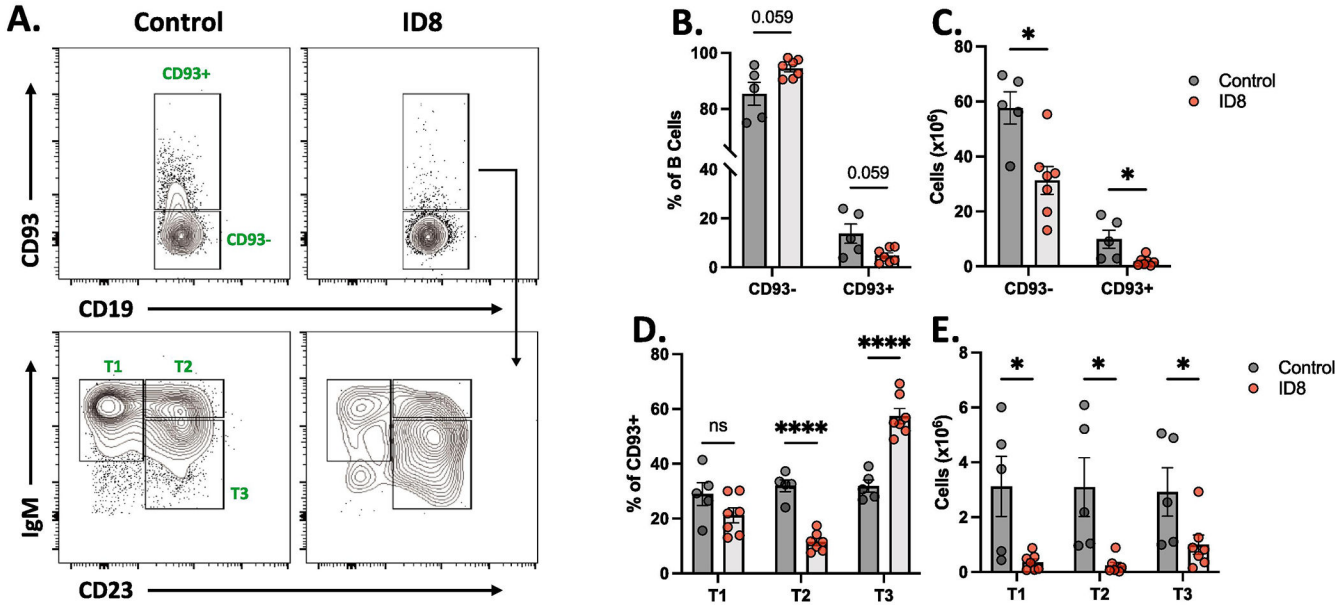

Fig. 3.

Transitional B cells are decreased in the spleen during ovarian cancer. (**A**) Representative flow cytometry profiles demonstrating gating strategy used to define transitional B cell subsets. (**B**) Frequency of B cells and (**C**) total numbers. (**D**) Frequency of transitional B cell subsets and (**E**) total numbers. FACS plots were pre-gated on single, lymphocytes, CD19+. Transitional B cells were gated based on CD93 FMO. Data sets compared using unpaired t tests *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

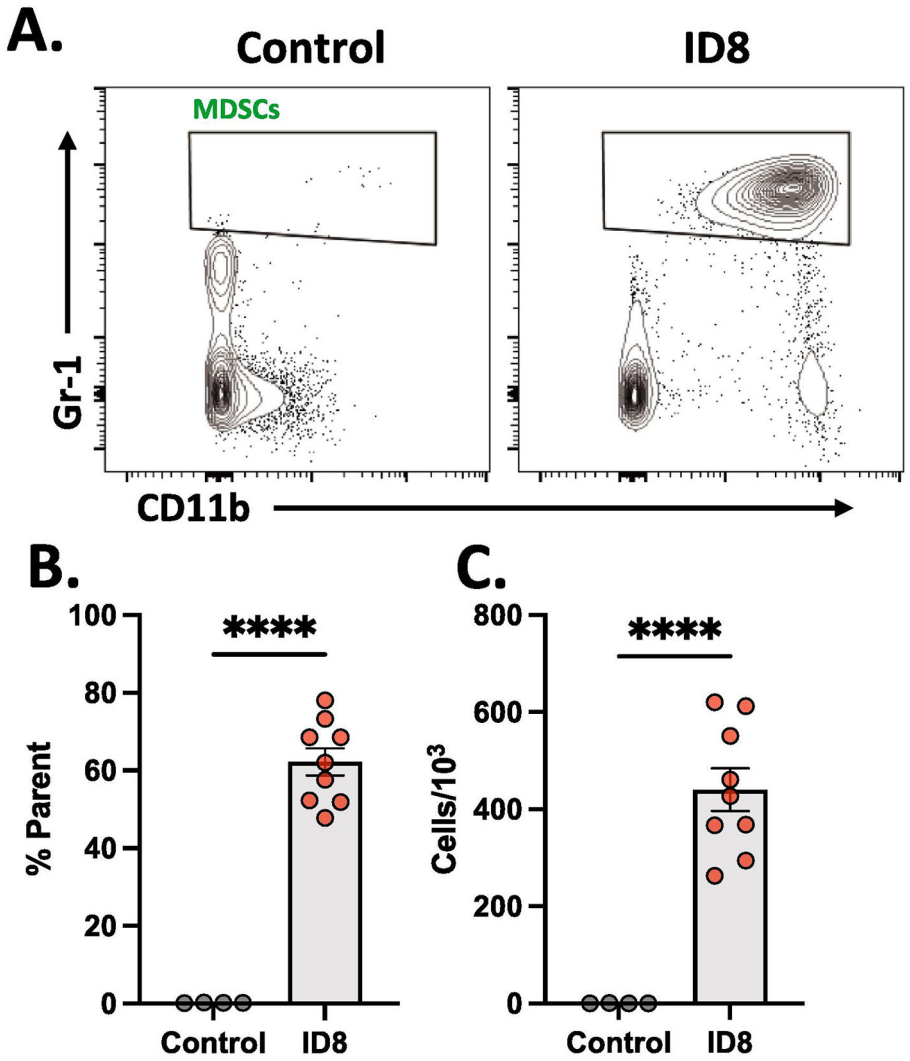

Fig. 4.

Myeloid cells are expanded during ovarian cancer. (**A**) Representative flow cytometry profiles of myeloid cells based on CD11b and Gr-1 expression. (**B**) Frequency of myeloid cells and (**C**) cells per thousand total cells. FACS plots were pre-gated on single, FWD SSC. Unpaired t test ****p<0.0001

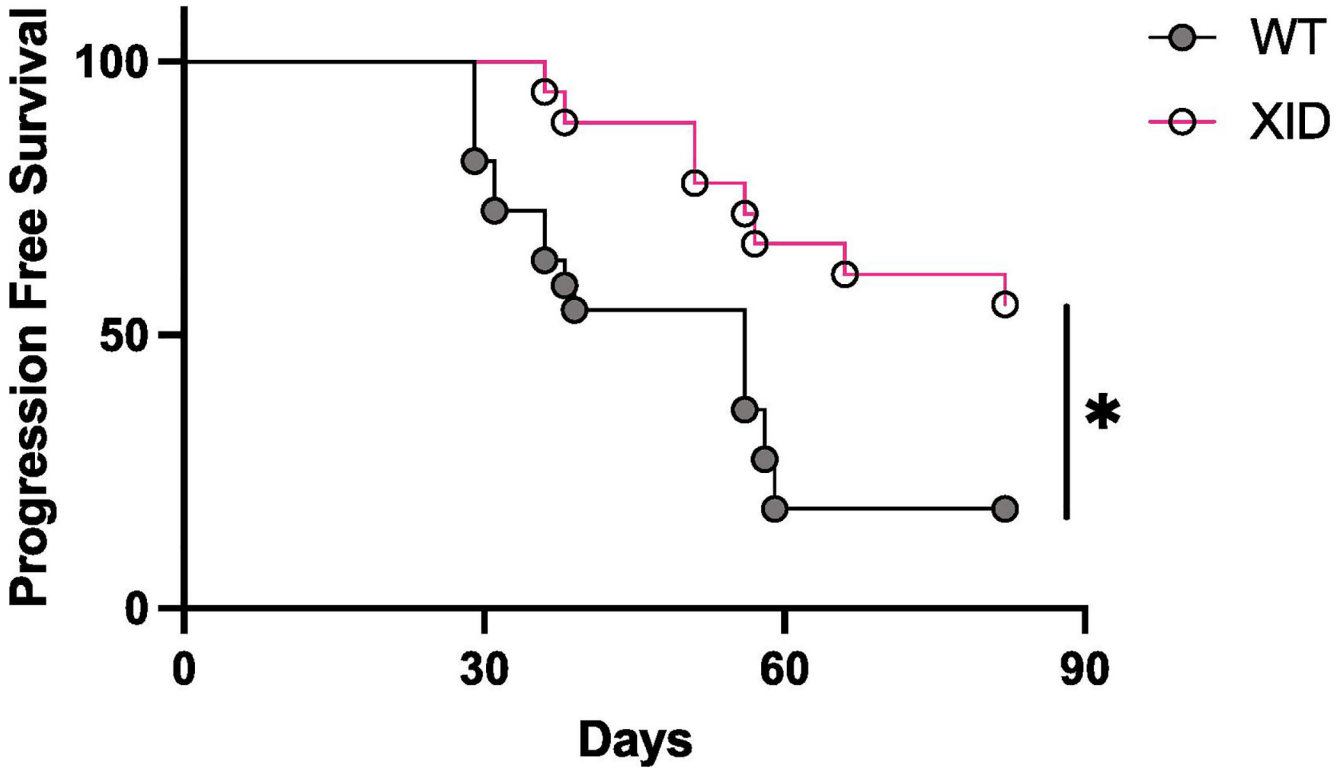

Fig. 5.

XID mice resist ovarian cancer. WT and XID mice were monitored for ascites development following ID8 administration. Kaplain-Meier survival analysis, *p<0.05

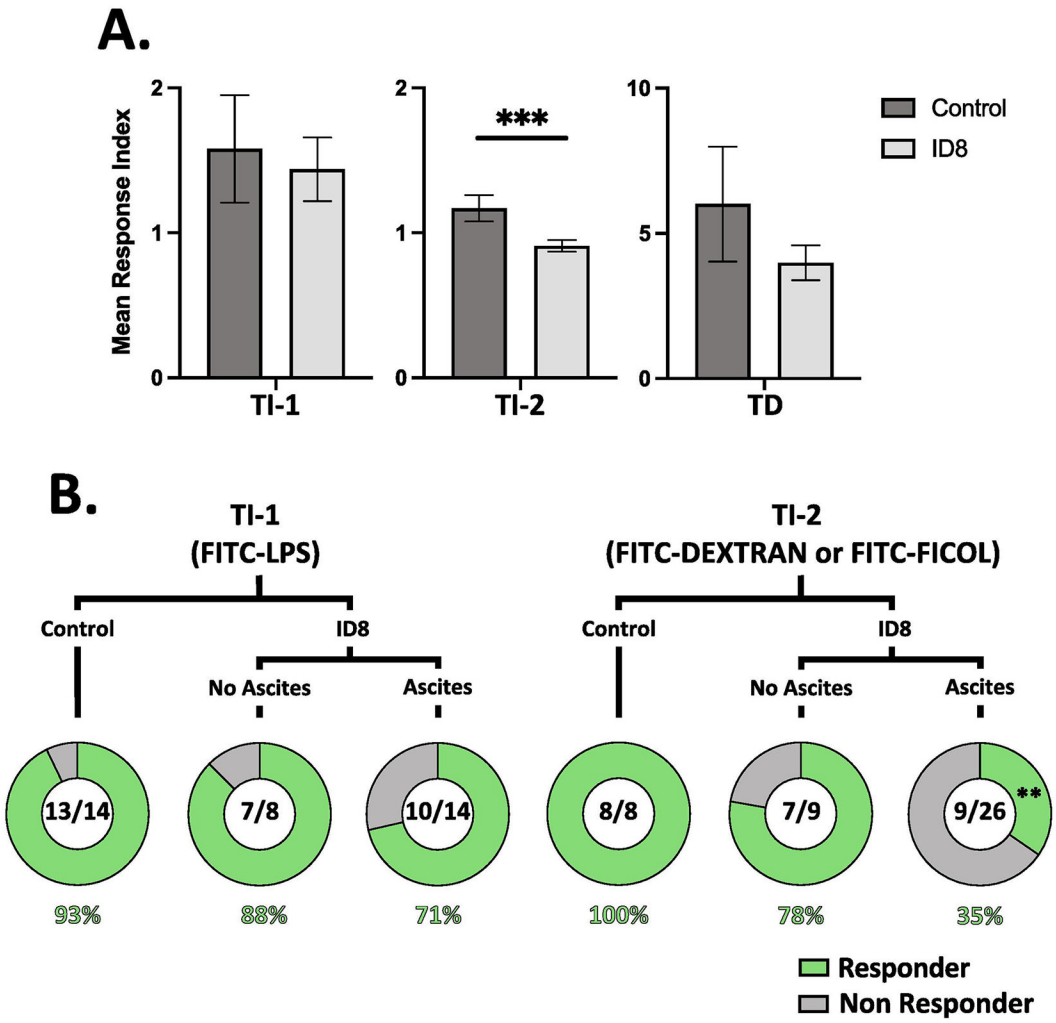

Fig. 6.

Humoral responses to TI-2 antigens are diminished during ovarian cancer. (**A**) Mean response index to TI-1, TI-2, and TD antigens in control mice or those with ovarian cancer. Each data set was compared by ANOVA ***p<0.001 (**B**) Frequency of responding mice in the TI-1 and TI-2 groups. Mice were immunized ip with FITC-LPS (TI-1), FITC-Dextran (TI-2), FITC- Ficoll (TI-2), or FITC-OVA (TD) and bled on day 6 (TI) or 12 (TD) post-immunization. Total anti-FITC immunoglobulin levels were measured by ELISA. Data were compared by the Chi-square test with Bonferroni correction **p<0.01.