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B lymphocytes exploit macroautophagy to capture cytoplasmic and nuclear proteins within autophagosomes. Fusion of autophagosomes with lysosomes and endosomes facilitates content proteolysis, with the resulting peptides selectively binding MHC class II (MHC II) molecules, which are displayed for recognition by T lymphocytes. Nutrient deprivation or stress amplified this pathway, favoring increased MHC II presentation of cytoplasmic Ags targeted to autophagosomes. By contrast, this stress diminished MHC II presentation of membrane Ags including the BCR and cytoplasmic proteins that use the chaperone-mediated autophagy pathway. Whereas intracellular protease activity increased with nutrient stress, endocytic trafficking and proteolytic turnover of the BCR was impaired. Addition of macronutrients such as high molecular mass proteins restored endocytosis and Ag presentation, evidence of tightly regulated membrane trafficking dependent on macronutrient status. Altering cellular levels of the cytosolic chaperone HSC70 was sufficient to overcome the inhibitory effects of nutritional stress on BCR trafficking and Ag presentation. Together, these results reveal a key role for macronutrient sensing in regulating immune recognition and the importance of HSC70 in modulating membrane trafficking pathways during cellular stress.

N utrition has a profound influence on host immunity, as seen by connections between immune deficiency and malnutrition (1). Protein malnutrition and serum protein levels are linked to increased susceptibility to infection (2, 3). In children, protein deprivation results in reduced Ab responses and B cell expansion with bacterial infection (4, 5). These studies indicate protein malnutrition impairs B and T cell–mediated immunity, although the molecular mechanisms remain poorly defined.

Induction of high-affinity, long-lasting humoral immunity depends on B and T cell interactions. Intracellular trafficking pathways play a key role in promoting B and T cell contact necessary for adaptive immune responses. Ags are delivered into the endosomal network by various transport routes including receptor-mediated uptake, macroautophagy (MA), and chaperone-mediated autophagy (CMA) (6, 7). Whereas endocytosis of exogenous Ags promotes the classical MHC class II (MHC II) Ag-presentation pathway, MA, bulk autophagy, and CMA, a selective form of autophagy dependent on HSC70, play critical roles in MHC II presentation of intracellular Ags (7).

To enhance Ag uptake, B cells express membrane-bound Ig, which associates with adapter proteins to form the BCR (8). Surface BCR, alone or complexed with Ag, is constitutively internalized into endosomes. In this study, cathepsins digest the BCR and Ags, yielding peptides for presentation by MHC II molecules (9, 10). MHC II are guided to endosomes by invariant chain, which is proteolytically released by cathepsins (11). A molecular editor, HLA-DM, then removes the invariant chain fragment CLIP from MHC II, facilitating antigenic peptide binding (12). The resulting MHC II–peptide complexes as well as some costimulatory molecules recycle from endosomes to the cell surface to engage T cells. Thus, trafficking of BCR–Ag complexes to endosomes promotes MHC II Ag presentation and enhances B and T cell interactions needed for Ab class-switching and affinity maturation (13, 14).

In response to some stresses, cells upregulate or downregulate transport pathways such as MA, CMA, and endocytosis to maintain homeostasis. During nutrient stress, cells initially upregulate MA coupled with later changes in CMA to promote survival and to salvage critical building blocks (15, 16). Whether alterations in the activity of these pathways during nutritional stress influence immune recognition has not been well explored.

In B cells, macronutrient stress induced by protein deprivation impaired BCR and Ag trafficking, altering MHC II Ag presentation. A shared requirement for the conserved heat shock protein HSC70 was demonstrated to maintain these B cell functions during nutrient stress. These studies reveal macronutrient sensing in B cells modulates Ag trafficking and presentation, further connecting host nutrition with adaptive immunity.

Materials and Methods

Cells and Ag presentation assay

Human B lymphoblastoid cell lines referred to as B cells or B lymphoblasts, including PriessGAD (PG) and FrevSMA, were maintained in IMDM with 10% FCS (17). Human peripheral blood B cells were isolated using Ficoll gradients and CD19 magnetic beads (Miltenyi Biotec) followed by culture in RPMI 1640 media with 10% FBS. Institutional approval for human blood collection was obtained for this study. For treatment without serum, cells were washed in HBSS and incubated in IMDM or RPMI 1640. PG cells were transduced to express influenza A matrix protein I (MP1) fused to the autophagy protein LC3 (plasmid a gift from C. Münn, University of Zurich) (18). Ectopic expression of human HSC70 in PG was previously described (19). T cells recognizing a BCR L chain (Igk 88–203) epitope, an epitope from MP1 (provided by David Canaday, Case Western Reserve University), an epitope from MHC class I (MHC I) H chain, or the glutamic acid decarboxylase (GAD)273–285 epitope, were maintained as reported (14). For analysis of Ag presentation, B cells were cultured in media ± serum or in serum-free media with BSA. B cells were then fixed and cocultured with epitope-specific T cells or pulsed with 10 μM peptide.

Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46202.

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Address correspondence and reprint requests to Dr. Janice S. Blum, Indiana University School of Medicine, 635 Barnhill Drive, MS 420, Indianapolis, IN 46202. E-mail address: jblum@iupui.edu

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Abbreviations used in this article: CHX, cycloheximide; CMA, chaperone-mediated autophagy; CQ, chloroquine; GAD, glutamic acid decarboxylase; HEL, hen egg lysozyme; MA, macroautophagy; MHC I, MHC class I; MHC II, MHC class II; MP1, influenza matrix protein I; PG, Priess GAD; TIR, transferrin receptor.

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for 6 h prior to incubation with epitope-specific T cells. T cell activation was detected using an IL-2 bioassay and FACS (20). To facilitate the comparison of results using human B lymphoblasts, the average value of T cell activation detected using control serum-cultured cells from three independent experiments was calculated and set to 1 in each study. For each experimental condition, the average T cell response was calculated from individual replicate experiments and plotted as a relative value compared with the average T cell responses observed using control cells, the latter referenced above as equal to 1.

**ImmunobLOTS**

Cell lysates from B cells cultured ± serum were resolved by SDS-PAGE for immunoblot analysis of LC3II (Cell Signaling Technology), BCR (Bio-source International), DR (DA6), invariant chain (PIN1.1), GAD (GAD65/67, Sigma-Aldrich), MP1 (Abd Serotec), or GAPDH (Miliopore). Densitometry using ImageJ (National Institutes of Health) was used to quantitate protein levels. Protein densitometry was normalized to the GAPDH or MHC II loading controls and the fold change determined relative to the control cells cultured with serum. For protein turnover, B cells were cultured in 10 μg/ml cycloheximide (CHX) ± serum prior to immunoblot analysis.

**MA flux**

Upon maturation and acidification of autophagosomes, LC3II protein with in these vesicles is degraded. B cells were cultured ± serum, ± 50 μg/ml chloroquine (CQ), harvested, and lysed for immunoblot and densitometry analysis. CQ is added to block autophagosomal degradation of LC3II. The relative increase in endogenous cellular LC3II levels detected with addition of CQ is a measure of MA flux. Basal cellular levels of LC3II/GAPDH were subtracted from LC3II/GAPDH levels accumulating with CQ treatment and normalized to serum-treated cells (21).

**Flow cytometry**

B cells were harvested after an incubation ± serum, fixed, and stained for surface markers: DR (BD Pharmingen), CLIP (BD Pharmingen), CD45R (eBioscience), transferrin receptor (TfR) (BD Pharmingen) or BCR (Jackson Immunoresearch Laboratories). To monitor surface levels of GM1 ganglioside, cells were incubated with 10 μg/10⁶ cells biotin-conjugated cholera toxin (Life Technologies) prior to staining with an anti–biotin-PE secondary Ab. For intracellular staining, B cells were permeabilized in 0.1% saponin prior to DM (BD Pharmingen) or BCR (Jackson Immunoresearch Laboratories) staining. Isotype-matched Abs were used as controls. The average mean fluorescence intensity of three independent experiments was determined for control serum-cultured cells with serum. The fold change relative to this average was graphed.

**Protease assays**

In this study, two separate assays were used to detect cellular cathepsin activity. In a static assay with cell lysates, cathepsin activity was monitored using plate-based fluorometry to detect cleavage of specific substrates: ZArgArgMec for cathepsin B, ZPheArgMec for cathepsin L, and ArgNMecHCl for cathepsin H (Biochrome). For real-time analysis of cathepsin activity within the endosomal network of live cells, a flow cytometric assay was used. In this study, Magic Red Cathepsin B and L Kits (Immunochrome Technologies) were used (23). After 11 h of treatment ± serum, B cells were incubated for 1 h with 6 μM membrane-permeable Magic Red protease substrates followed by flow cytometric analysis to detect substrate cleavage.

The Protease-Glo Chymotrypsin-like Cell-Based Assay (Promega) was used according to the manufacturer’s instruction to assess protease activity in control cells cultured with serum and after 6- or 12-h serum deprivation (24). The average protease activity from three independent experiments for control cells fed with serum was determined, and the fold change relative to this average was graphed.

**Endocytosis assay**

Specific endocytosis of the BCR, MHC II, and the Tf was monitored by biotinylating B cell surface proteins using EZ-link Sulfo-NHS-SS-Biotin (Thermo Scientific) (25). Biotin-labeled cells were incubated 30 min at 37°C in media ± serum or in serum-free media with BSA. After the incubation, a glutathione solution was used to remove residual biotin label from surface proteins. Cells were lysed and cell-surface protein endocytosis determined using ELISA plates coated with anti-human IgG Ab (Jackson Immunoresearch Laboratories, used at 2 μg/ml) for the BCR, or at 23.1 Ab (L. Wicker, used at 1 μg/ml), or anti-Tf B3/25 Ab. Endocytosis was graphed as a percent of the total surface expression for each protein.

**Subcellular fractionation**

To determine if serum starvation altered GAD intracellular localization, membrane and cytoplasmic fractions were separated using a method previously published (26). PG cells were cultured with or without serum for 12 h, harvested, and lysed using the Balch homogenizer. Differential ultracentrifugation was then used to isolate membrane and cytosolic fractions for immunoblot analysis.

**Macronutrient supplementation of media**

Serum was fractionated using Centricron concentrators with cutoffs of 30 and 10 kDa according to the manufacturer's instructions (Amicon). Macromolecules retained by the 30-kDa filter (>30 kDa fraction) and molecules flowing through filters were used as <30 and <10 kDa fractions, respectively. Cells were cultured in IMDM media supplemented at 10% with the various serum fractions for 12 h before harvest.

Alternatively, cells were cultured in serum-free media supplemented with defined macromolecules including 1% (w/v) BSA, OVA, hen egg lysozyme (HEL), or dextrans (70, 40, or 10 kDa, respectively). After 12 h, these cells were analyzed by flow cytometry.

**Osmolarity and viscosity**

Analysis of the osmolarity and viscosity of media samples with and without serum, BSA or dextran addition was conducted by a commercial laboratory ACTA using United States Pharmacopeia 37/NF 32 methodology (Burbank, CA).

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**FIGURE 1.** Macronutrient deprivation alters endogenous Ag presentation and degradation. (A) B cells were grown ± serum (Cont.) or - serum, and MHC II presentation of MP1 epitopes was monitored. (B) To measure MA flux, B cells were grown ± serum and treated with CQ to stabilize and detect endogenous LC3II accumulation in autophagosomes. MA flux in Cont. cells was normalized and set to 1 (dotted line). (C) B cells were grown ± serum and MHC II presentation of endogenous BCR epitopes detected using epitope-specific T cells. (D) B cells were cultured ± serum for 12 h, fixed, and incubated for 6 h with 10 μg/M BCR peptide prior to incubation with T cells and detection of IL-2. (E) B cells were grown for 12 h ± serum and changes in the expression of surface HLA-DR (MHC II) and intracellular HLA-DM detected by flow cytometry. HLA-DM function was assessed by monitoring MHC II–CLIP surface levels. Protein levels in Cont. cells were set to 1 (dotted line) and the relative expression of DR, CLIP, or DM during serum deprivation indicated. (F) B cells were grown ± serum for 12 h and lysed for immunoblot analysis of MHC II, invariant chain (Ii Chain), and GAPDH. (A and C) One-way ANOVA, multiple comparison. (B) Student t test, multiple comparison with Cont. cells. *p < 0.05, **p < 0.01.
Coimmunoprecipitation

B cells were cultured in media ± serum or in serum-free media supplemented with 1% (w/v) BSA for 12 h. Cells were lysed in 10 mM Tris (pH 7.4), 150 mM NaCl, and 1% N-octyl-β-D-glucopyranoside with protease inhibitor (Sigma-Aldrich) for 20 min on ice. Lysates were centrifuged at 14,000 rpm for 10 min. Normal rabbit serum (1:1000) and Protein G-Sepharose were added to lysate at 4˚C for 30 min as a preclear step followed by removal of the Sepharose by centrifugation. GAD Ab (GAD6; Sigma-Aldrich) or an isotype-matched control Ab were added to coprecipitate associated proteins. These Ag–Ab complexes were resolved on 10% SDS-PAGE and analyzed by immunoblotting. The ratio of HSC70/GAD was set to 1 in control serum-treated cells, and the fold change relative to this ratio was graphed for each experimental condition.

Statistics

Statistics were determined using GraphPad Prism 6.0 (GraphPad Software). Data depict mean from three or more independent experiments ± SEM. The p values <0.05 were considered statistically significant with *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

Results

Nutrient deprivation alters MHC II Ag presentation

To elucidate the effects of macronutrient deficiency on MHC II presentation, B cells were cultured in serum-free media rich in amino acids, simple carbon sources, and vitamins but lacking complex macromolecules. In vivo and in vitro, cellular deprivation of serum proteins is known to induce MA, a process by which LC3II-decorated autophagosomes engulf cytoplasmic molecules for degradation and, in some cases, immune recognition. MHC II presentation of epitopes derived from a cytoplasmic Ag, influenza MP1 that is targeted to autophagosomes by linkage to LC3, was increased in cells grown in serum-free media compared with serum-treated cells (Fig. 1A). Cellular levels of MA or the MA flux was monitored by tracking the expression of the endogenous LC3II protein in response to nutrient stress with and without CQ. As expected, MA flux was amplified in cells grown without serum (Fig. 1B). Although MHC II (HLA-DR)
surface levels and MHC II presentation of a synthetic peptide derived from the BCR were unchanged by serum deprivation. MHC II presentation of endogenous BCR epitopes was significantly inhibited (Fig. 1C–E). HLA-DM levels and editing of MHC II–CLIP complexes were unchanged by serum deprivation (Fig. 1E). Furthermore, cellular levels of MHC II and invariant chain were overall unchanged (Fig. 1F, Supplemental Fig. 1A). Thus, serum deprivation in B cells stimulated Ag presentation by MA while reducing MHC II display of self-epitopes derived from a membrane Ag the BCR.

Nutrient deprivation alters Ag degradation

To elucidate the mechanism by which serum deprivation altered epitope selection, MP1 and BCR protein expression was assessed. Whereas cellular MP1 protein levels dropped with serum deprivation (Fig. 2A), BCR protein levels dramatically increased (Fig. 2B). To determine whether these alterations were due to changes in Ag synthesis or degradation, B cells were treated with a protein synthesis inhibitor, CHX. MP1 protein levels decreased quickly with serum deprivation regardless of CHX treatment, consistent with MA-induced degradation (Fig. 2A). Conversely, BCR protein levels rose irrespective of CHX, consistent with impaired BCR degradation (Fig. 2B). Transcripts for BCR and MHC II components were unchanged by serum deprivation, further pointing to perturbations in cellular protein-degradation pathways (Supplemental Fig. 1B, 1C).

To determine if disruption of BCR degradation was due to altered endosomal and lysosomal protease activity, cathepsin B, L, and H activity was determined in lysates from B cells cultured with or without serum. Serum deprivation led to a significant increase in total cellular cathepsin B and L activity, but cathepsin H remained unchanged (Supplemental Fig. 1D). Furthermore, a real-time flow analysis of cathepsin proteolysis using membrane-soluble substrates

in living B cells revealed increased endo/lysosomal cathepsin B and L activity in response to serum deprivation (Fig. 2C, 2D). Furthermore, serum deprivation increased cathepsin B and L activity in freshly isolated human peripheral blood B cells from multiple donors (Fig. 2E). The reduced BCR turnover in the presence of increased cathepsin activation in this analysis pointed to possible alterations in BCR trafficking during serum macronutrient depletion.

Nutrient deprivation impairs endocytosis and trafficking of the BCR

Analysis of BCR trafficking revealed a 2-fold increase in BCR surface levels in B cells cultured without serum (Fig. 3A). Total BCR expression increased with serum deprivation, along with the ratio of surface/total BCR expression (Fig. 3B), consistent with an accumulation of cell-surface BCR. Surface BCR distribution increased more dramatically in peripheral blood B cells incubated without serum (Fig. 3C, 3D). Similarly, serum deprivation significantly enhanced surface levels of the TIR, which also uses the clathrin-mediated endocytosis pathway (Supplemental Fig. 1E). Interestingly, serum deprivation did not alter surface expression of CD45R nor the ganglioside GM1, neither of which use clathrin-mediated endocytosis for internalization (Supplemental Fig. 1F, 1G). Specifically, internalization or endocytosis of the BCR was significantly impaired in response to serum deprivation of B lymphoblasts (Fig. 3E) as well as in peripheral blood B cells (Fig. 3F). To further assess the effect of serum deprivation on clathrin-mediated endocytosis, internalization of surface MHC II and TIR was monitored in peripheral blood B cells. Endocytosis of these molecules was slightly impaired with cellular serum deprivation (Fig. 3F). These studies pointed to a role for serum nutrients in regulating clathrin-mediated endocytosis.

**FIGURE 4.** Exogenous macromolecules restore BCR trafficking and MHC II Ag presentation. (A) BCR surface expression was determined in B cells treated for 12 h + serum (Cont.), − serum, or in serum-free media supplemented with different fractions of serum (>30, <30, or <10 kDa). Cont. cell BCR surface expression was normalized and set to 1 (dotted line). (B) BCR surface expression was determined in B cells treated for 12 h ± serum or in serum-free media supplemented with BSA or 70 kDa dextran. Cont. cell BCR surface expression was normalized and set to 1 (dotted line). (C) BCR surface levels were determined in B cells cultured ± serum or in serum-free media supplemented with various sized proteins: 14 kDa HEL, 45 kDa OVA, or 68 kDa BSA for 12 h. (D) BCR endocytosis was monitored in B cells cultured ± serum or in serum-free media supplemented with BSA. (E) B cells were cultured ± serum or in serum-free media supplemented with BSA for 12 h, harvested, and fixed for flow cytometric analysis of MHC II surface expression. MHC II presentation of BCR epitopes (F), MHC I epitopes (G), and MP1 epitopes (H) was monitored in B cells treated ± serum or in serum-free media supplemented with BSA for 12 h. (A–C) Student t test, multiple comparison. *Denotes p value as compared with Cont. cells (dotted line); # denotes a one-way ANOVA, multiple comparison. (D–H) One-way ANOVA, multiple comparison. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
Macronutrients restore trafficking and Ag presentation

Serum is macronutrient rich, containing proteins, lipids, and complex carbohydrates. To determine if macronutrients played a role in alterations in BCR trafficking, serum components were fractioned based on molecular mass. Internalization of BCR was reduced with serum deprivation or upon cultivation of B cells with serum components <10 kDa. Serum fractions with higher molecular mass components (>10 kDa) were more effective at restoring surface BCR levels (Fig 4A).

To determine if specific macromolecules were responsible for alterations in BCR trafficking, the effects of protein and carbohydrate supplementation on serum-deprived B cells was assessed. Supplementation of B cell culture media with the protein BSA (68 kDa) or with the complex carbohydrate dextrans (70 kDa) was sufficient to normalize BCR surface levels (Fig. 4B). The osmolarity and viscosity of the media alone or supplemented with the various macromolecules was monitored and shown to not change (Supplemental Fig. 1H, 1I). Consistent with the serum fractionation, larger macronutrients or macromolecules were more effective at restoring surface BCR levels, as shown by supplementing media with different sized proteins: BSA, OVA (45 kDa), or HEL (14 kDa) (Fig. 4C) or dextrans (Supplemental Fig. 1J). Denatured or fatty acid-free BSA also restored surface BCR levels, suggesting protein conformation or bound fatty acids were not critical (Supplemental Fig. 1K). Whereas BSA supplementation did not alter MHC II surface expression, this macronutrient restored BCR internalization and MHC II presentation of BCR epitopes (Fig. 4D–F). Furthermore, MHC II presentation of an epitope derived from MHC I H chain, another membrane Ag that uses clathrin-mediated endocytosis, was significantly impaired during serum deprivation and could be rescued by macronutrient supplementation (Fig. 4G). BSA supplementation only marginally reduced MHC II presentation of MP1 epitopes (Fig. 4H). Thus, B cell sensing of exogenous macronutrients effectively altered membrane internalization, impacting BCR trafficking and MHC II peptide selection.

Macronutrient deprivation impairs trafficking and MHC II presentation of cytoplasmic Ags that are targets of CMA

To further elucidate the mechanism by which macronutrient deprivation altered MHC II peptide presentation, the effect of serum deprivation on MHC II presentation of GAD, a cytosolic Ag that uses CMA to encounter MHC II, was monitored in B cells. Interestingly, GAD Ag mirrored the results obtained with the BCR epitope. Whereas MHC II presentation of GAD peptide was unaltered by serum deprivation, MHC II presentation of an epitope derived from the endogenous GAD Ag was significantly impaired during macronutrient deprivation (Fig. 5A, 5B). Similar results were observed for MHC II presentation of a second CMA substrate, SMA (Supplemental Fig. 1L). Exogenous BSA supplementation could prevent these changes in GAD presentation (Fig. 5A). Similar to the BCR, GAD protein levels rose during serum deprivation irrespective of CHX, consistent with impaired GAD degradation (Fig. 5C). GAD is degraded by the proteasome as well as lysosomal cathepsins (27). Serum deprivation induced proteasome activity (Fig. 5D). GAD, although a cytosolic protein, can be palmitoylated and associate with membranes (28). Serum deprivation altered the subcellular distribution of GAD, reducing the relative levels of free cytosolic GAD and increasing the levels of membrane-associated...
GAD (Fig. 5E, 5F). During CMA, select proteins are translocated from the cytosol to the lumen of the lysosome by the transmembrane protein LAMP2A. LAMP2 protein and mRNA levels were assessed, revealing LAMP2 levels were increased by cellular serum deprivation, suggesting the expression of this transporter was not limiting (Fig. 5G, 5H) (29). Although epitopes from GAD and the BCR use seemingly disparate pathways to encounter MHC II presentation, serum deprivation altered their trafficking, degradation, and MHC II presentation in similar ways.

**Macronutrient deprivation impairs HSC70 association with cytoplasmic GAD Ag**

HSC70 is required for both clathrin-mediated endocytosis and CMA, the pathways required for BCR and GAD presentation by MHC II, respectively. To assess whether serum deprivation altered HSC70 activity, GAD association with HSC70 was monitored in B cells. GAD association with HSC70 in control serum-fed cells or BSA-supplemented cells was similar, yet macronutrient deprivation significantly reduced the association of GAD and HSC70 (Fig. 6A, 6B). Total cellular HSC70 protein expression remained unaltered by macronutrient deprivation (Fig. 6C, 6D). These data suggest macronutrient deprivation may alter the subcellular distribution and function of HSC70.

**Ectopic expression of HSC70 overcomes the effects of macronutrient deprivation in B cells**

To assess the role of HSC70 during serum deprivation, B cells were transduced to ectopically express cytoplasmic HSC70 (Fig. 7A). Ectopic expression of HSC70 restored GAD association with HSC70 during macronutrient deprivation (Fig. 7B). Though this ~2-fold overexpression of HSC70 did not alter expression of MHC II components, it restored MHC II presentation of epitopes derived from GAD, BCR, and MHC I during nutrient deprivation (Fig. 7C, 7D, Supplemental Fig. 2A–D). Furthermore, ectopic expression of HSC70 reduced MHC II presentation of the MPM1 epitope in serum-deprived cells (Fig. 7E). Consistent with this, ectopic expression of HSC70 prevented MA induction during serum deprivation, though surprisingly, media supplementation with BSA failed to alter cellular MA flux (Supplemental Fig. 2E). Finally, ectopic HSC70 expression restored the trafficking of the BCR, TfR, and GAD in B cells, as seen by the normalization of surface BCR and TfR expression, and restored the distribution of cytosolic and membrane GAD during macronutrient deprivation (Fig. 7F–H, Supplemental Fig. 2F). Restoration of Ag trafficking with increased HSC70 expression in B cells demonstrates a key role for this chaperone in modulating cellular stress and intracellular protein transport.

**Discussion**

In the current study, macronutrient deprivation was shown to directly impact epitope selection and MHC II Ag presentation by disrupting clathrin-mediated endocytosis and perturbing multiple routes of autophagy. Alterations in intracellular Ag trafficking compromised MHC II presentation of epitopes derived from the BCR, MHC I, and the cytoplasmic Ags GAD and SMA. By contrast, MHC II display of epitopes derived from Ags targeted to autophagosomes increased with enhanced cellular MA during macronutrient deprivation. These data suggest coordinate regulation during cell stress of the pathways that deliver membrane and cytoplasmic Ags to the endosomal network for processing and MHC II presentation.

MA and clathrin-mediated endocytosis share several key chaperones, including mAtg9, TBC1D5, and AP2, suggesting potential coordinate regulation of these intracellular transport pathways in different cells, although this has not been examined in the context of immune cells (30–32). The current study reveals a novel role for HSC70 in controlling these pathways during macronutrient stress of B lymphocytes. HSC70 is a constitutively expressed chaperone critical for clathrin-mediated endocytosis, proteasome degradation, as well as the selective capture of proteins for translocation into lysosomes via CMA (33, 34). HSC70 guided transit of proteins to the proteasome and CMA appears widely conserved in neural cells as well as immune cells (19, 27, 35, 36). Studies from our laboratory demonstrated HSC70 binds to select proteins such as GAD Ag, delivering them for proteolytic processing and MHC II presentation (19, 27). Furthermore, in fibroblasts, HSC70 association with autophagosomes may play a role in the clearance of cytoplasmic protein aggregate complexes by MA (37, 38). Intracellular competition for HSC70 was recently observed, as induction of MA by protein aggregates depleted chaperone reserves, impairing clathrin-mediated endocytosis (38). Our data indicate during macronutrient stress in B lymphocytes, these trafficking pathways compete for conserved chaperones such as HSC70, with upregulation of MA and proteasome activity at the expense of endocytosis and CMA. This may favor the presentation of intracellular pathogens or self-Ags sequestered in autophagosomes, while limiting immune recognition of endocytosed Ags. Furthermore, studies in this paper are the first to suggest a role for HSC70 in regulating stress-induced MA. Thus, HSC70 plays a key role selectively regulating endocytosis and autophagy during nutritional stress, impacting Ag presentation and B–T cell interactions.

Multiple mechanisms likely contribute to compromised adaptive immune responses in malnourished individuals (5, 39). For example, zinc deficiency is linked to protein malnourishment and can impair T cell responses, suggesting a role for this micronutrient in regulating host immunity (1, 40). Furthermore, studies have revealed protein deprivation reduces the capacity of macrophages to phagocytosis bacteria in vivo and in nonimmune cells disrupts transferrin receptor endocytosis, indicative of more global effects of nutritional stress on vesicular sorting (32, 41). Whether protein malnourishment impacts APCs such as B cells had not...
FIGURE 7. Ectopic expression of HSC70 averts disruptions in Ag trafficking during macronutrient deprivation. (A) The PG B lymphoblasts were transfected to ectopically express HSC70 (PG70). PG and PG70 cells were lysed for immunoblot analysis of HSC70 and GAPDH expression. Densitometry was used to quantify relative ratio of HSC70 to GAPDH expression in these cells. (B) PG70 cells were cultured + serum (Cont.) or − serum for 12 h. Lysates from these cells were incubated with a GAD-specific Ab or an isotype-matched Ab overnight to immunoprecipitate (IP) GAD. Immunoprecipitated proteins were resolved using SDS-PAGE, immunoblotted for HSC70 and GAD, and densitometry was used to quantify relative amounts of HSC70 bound to GAD. PG and PG70 cells were grown ± serum and tested for MHC II presentation of endogenous GAD epitopes (C), endogenous BCR epitopes (D), or MP1 epitopes (E). (F) Flow cytometry of PG and PG70 cells cultured ± serum for 12 h prior to detection of BCR surface levels. (G and H) PG70 cells were grown ± serum or in media supplemented with BSA for 12 h. These cells were lysed and cytoplasmic and membrane fractions isolated. Fractions were resolved by SDS-PAGE and immunoblotted for GAD and, as a loading control, GAPDH (cytoplasmic) or MHC II (membrane). Densitometry was used to quantify GAD levels relative to the loading control protein. (A) Student t test. (C–H) Student t test, multiple comparisons correction. *p < 0.05.

been tested. The current study now reveals a novel link between exogenous macronutrient levels and Ag trafficking in B cells, offering further explanation for reduced Ab responses and impaired T cell–mediated immunity observed in protein-malnourished individuals (4). These observations also point to the exquisite sensitivity of lymphocytes to nutrient stress.

In summary, connections between cellular endocytic and autophagic pathways shape MHC II Ag presentation to influence adaptive immunity. In this study, macronutrient availability was shown to upregulate MA flux and impair the ability of B cells to internalize cell-surface Ag receptors, impacting MHC II Ag presentation. These data revealed HSC70 as a connection between routes of Ag delivery, suggesting coordinated regulation of these pathways may influence MHC II epitope selection. Together, these findings reveal a previously unknown connection between macronutrient sensing and Ag trafficking within lymphocytes, which may contribute to impaired adaptive immune response in protein malnourished individuals.

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