diseases. At remission antithrombin increases while endothelial cell activity (VWF and FVIII) remains high. These observations indicate that regulation of coagulation and fibrinolysis contributes to pathogenesis of AAV and offer surrogate markers for disease activity.

**Further readings**

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**P188**
Galactosylation and sialylation of anti-proteinase 3 antibodies in granulomatosis with polyangiitis is associated with increased inflammatory cytokines
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**Introduction**—Interaction between immunoglobulin G (IgG) and its receptors is determined by glycosylation of Asn297 on the antibody’s Fc portion. Previous in vitro studies have demonstrated that 1 cell cytokines influence glycosylation status of immunoglobulin produced by B cells.

**Methods**—Serum was collected from 38 healthy controls (HC) and 49 patients with GPA and anti-proteinase 3 (PR3) antibodies. IgG was purified from serum using Protein A-Sepharose beads. Anti-PR3 specific IgG (aPR3) was isolated using a PR3 specific ELISA plate. Glycosylation of IgG was determined by mass spectrometry. Clinical data was collected prospectively on 10 GPA patients (GPaC) and serum cytokines were measured by multiplex assay. All patients gave informed consent.

**Results**—aPR3 was only isolated from patients with GPA and was predominantly IgG1. Agalactosylated total IgG1 was more common in GPA than HC (52% vs 29%; P < 0.001) and agalactosylation was even more common in aPR3 specific IgG1 (59%; P = 0.006). Galactosylation (Gn) and sialylation (Sn) were lowest in aPR3, low in GPA IgG1 and highest in HC IgG1. Although reduced Gn and Sn were associated with GPA within the 10 GPaC patients there were positive correlations between cytokine concentrations, aPR3 (but not IgG1) Gn and time to remission (table I). There were less significant correlations between aPR3 Sn and cytokines. There were no correlations between Gn or Sn of GPA IgG1 and aPR3. There was no correlation between Gn or Sn and Birmingham Vasculitis Activity Score.

**Discussion**—Our data suggests that although reduced Gn and Sn is associated with GPA compared to HC the proinflammatory cytokine environment in patients drives increased Gn of autoantibodies and is associated with an increased time to remission indicating that 1 cell activity may be important in determining autoantibody glycosylation and time to remission.

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**P189**
LAMP-2, a potential novel receptor on human monocytes derived dendritic cells (MDDC)
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**Introduction**—Pauci-immune-focal-necrotizing-glomerulonephritis (FNGN) is an inflammatory disease associated with autoantibodies (ANCA). Lysosomal-membrane-protein 2 (LAMP-2) is a recently defined target of circulating autoantibodies in addition to the more commonly known targets [1]. LAMP-2 is a type-2 membrane-protein that is used as lysosome and late endosomal marker and with essential roles in chaperone mediated autophagy and antigen presentation [2]. LAMP-2 is also present on the cell-surface although its role was never studied. Here, we focus on the role of LAMP-2 as a specific endocytic receptor acting on freshly isolated monocytes and iDCs with possible implication in antigen presentation.

**Methods**—Monocytes Derived Dendritic Cell and activation CD14+ monocytes are isolated from PBMCs using MACS, culture along 7 days with GM-CSF and IL-4. The activation status is checked using CD80, CD83 MHCI by FACS. Uptake experiment DCs are co-cultured with anti LAMP-2 antibody (H4B4) and then stained intracellular detected via FACS and IF. Co-localization confocal microscopy imaging is used to track the intracellular route of H4B4.

**Results**—LAMP-2 was detected on the surface of immature MDDC both by FACS and confocal microscopy and likewise other immune endocytic receptor its expression there increased after maturation. The monoconal anti-LAMP-2 antibody, H4B4 bound to LAMP-2 on the surface of MDDC and was rapidly and specifically internalized when compared to control antibodies in the presence of Fc receptor blockade. Uptake was absent in MDDC from an individual with genetic LAMP-2 deficiency. As expected, MDDC LAMP-2 and HLA-DR localized to partially overlapping compartments in immature DC but there was no co-localization with HLA-DM. However, confocal microscopy showed that anti LAMP-2 antibodies transits into a HLA-DM positive compartment 1 hour after ligating LAMP-2 on the cell surface. Co-localization is no longer detectable after 3 hours. Stimulation of immature MDDCs with anti-LAMP-2 antibody can substitute for LPS to promote activation in the presence of IFN-γ indicated by up-regulation of CD80/CD83. Finally, the uptake of FITC-dextran particle is higher in H4B4 stimulated DCs respect LPS stimulated DCs [3].

**Discussion**—Circulating autoantibody directed against LAMP-2 cause direct activation of DCs and increase dextrin uptake, therefore it is possible that this antibody are responsible for breaking of the tolerance leading to ANCA production. Biological implication implies the possibility that H4B4 mimic other immunogenic molecules.

### Table I

**Correlations between serum cytokine concentrations, aPR3 Gn and time to remission**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>% aPR3</th>
<th>P value</th>
<th>Time to remission</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>0.84</td>
<td>0.002</td>
<td>0.69</td>
<td>0.028</td>
</tr>
<tr>
<td>IL-1b</td>
<td>0.65</td>
<td>0.043</td>
<td>0.79</td>
<td>0.006</td>
</tr>
<tr>
<td>IL-12</td>
<td>0.7</td>
<td>0.025</td>
<td>0.77</td>
<td>0.009</td>
</tr>
<tr>
<td>IL-15</td>
<td>0.98</td>
<td>&lt; 0.001</td>
<td>0.69</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Conclusion.—In conclusion, this data suggests that when LAMP-2 is on the membrane acts as a specific receptor for internalization of extracellular molecules. After internalization, the ligand can be found into the MIIC causing as well the maturation of DCs up-regulating CD80/83.

References

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P190
Autoantibodies for several antigens in neutrophil cytoplasm other than PR3 and MPO also promote release of NETs from neutrophils
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Introduction.—Neutrophil extracellular traps (NETs) were first described as web-like structures that trap and neutralize microbes at sites of infection. NETs are comprised of chromatin components and neutrophil cytoplasmic proteins, and it has been reported that NETs are involved in autoimmunity such as SLE or ANCA-associated vasculitis (AAV). It has also been reported that autoantibodies themselves for important cytoplasmic autoantigens of neutrophils such as MPO and PR3 induce NET production. However, the role of other neutrophil cytoplasmic antigens for the production of NETs is unclear. We investigated how autoantibodies for these antigens are involved in NETs productions.

Methods.—Human peripheral blood neutrophils were obtained from healthy donors. Neutrophils were cultured with PMA (control) or PMA plus several kinds of anti-neutrophil cytoplasmic antigens for 3 hours. Cells were stained with Hoechst 3,3342, Sytox Green and anti-MPO antibody. The percentage of NETs producing cells and the quantitation of nuclear decondensation were analyzed using Image J software. We distinguished between cells that released NETs fiber and cells that just died of NETosis.

Results.—Anti-MPO antibody strongly promoted both NETosis induction and release of NETs fiber. Although anti-PR3 antibody promoted NETosis induction, it did not increase the release of NET fiber. Antibody for cathespin G, found in the azurophil granule, also promoted NETosis induction only. There were some other antibodies, such as anti-lactoferrin and anti-neutrophil elastase, that promoted both NETosis and release of NETS fiber.

Discussion.—It has been thought that ANCA has an important role in the pathogenesis of AAV partly because ANCA promote NETs production by neutrophils at sites of vasculitis. We showed that not only anti-MPO and anti-PR3 but also antibodies for other neutrophil cytoplasmic antigens promote NETs production. The mechanism these antibodies induce NETosis and what they do in the pathogenesis of AAV are to be elucidated.

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P191
Antibodies to LAMP-2 alter lysosome function and attenuate chaperone-mediated autophagy in human macrophages
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Introduction.—Lysosome-associated membrane protein-2 (LAMP-2) is membrane protein that traffics from the cell surface to lysosomes. It maintains lysosomal integrity and is rate limiting for chaperone-mediated autophagy (CMA). Anti-LAMP-2 antibodies are frequent in patients presenting with ANCA-associated vasculitis (AAV) [1,2]: whether they affect LAMP-2 function is unknown. Here, we determine whether antibodies to LAMP-2 compromise lysosome function and affect cell survival of human macrophages.

Methods.—Human THP-1 cells and monocyte-derived macrophages (MDM) incubated alone or together with monoclonal antibodies to LAMP-2 (H4B4) or isotype control (CD4). IgG uptake was measured by confocal microscopy and Western blotting (WB); and viability and apoptosis by trypan blue and caspase-3. Lysosomes were purified (lysosome kit) and their proteins quantified by WB and fluorescence. CMA was assessed by lysosomal acquisition of H5C70 and import of cytoplasmic proteins.

Results.—H4B4 was selectively taken up by THP-1cells and significantly increased apoptosis and cell death after 24 hours. It accumulated in lysosomes and significantly reduced LAMP-2 but not LAMP-1: CD4 did not. Lysosomal H5C70 was also reduced and correlated with LAMP-2 and implying a reduction in basal CMA. H4B4 abrogated of the stress-induced CMA response as shown by measuring the CMA substrate GAPDH and the reduced import of cytosolic proteins. H4B4 also increased lysosomal pre-pro-cathepsin D but not mature cathepsin D, suggesting lysosomal integrity was also affected. H4B4 had similar on MDM.

Discussion.—H4B4 is specifically taken up by macrophages and traffics to lysosomes where it reduces membrane LAMP-2 and disrupts function. It markedly inhibits basal CMA and abrogates its stress-induced augmentation. There are clear implications for macrophage function is obvious not least because CMA is critical for antigen presentation to T cells.

Conclusion.—H4B4-triggered CMA dysfunction provides the relevant molecular mechanism for cell death by antibodies to LAMP-2.

References

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P192
Urinary HMGB1 levels are associated with CD4+ T-cells in urine in patients with ANCA-associated vasculitis and active nephritis
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Introduction.—High prevalence of autoantibodies to hLAMP-2 in anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV) is strongly associated with the presence of active nephritis. The role of LAMP-2 in the pathogenesis of AAV is unclear. Here, we determine whether antibodies to LAMP-2 compromise lysosome function and affect cell survival of human macrophages.

Methods.—Human THP-1 cells and monocyte-derived macrophages (MDM) incubated alone or together with monoclonal antibodies to LAMP-2 (H4B4) or isotype control (CD4). IgG uptake was measured by confocal microscopy and Western blotting (WB); and viability and apoptosis by trypan blue and caspase-3. Lysosomes were purified (lysosome kit) and their proteins quantified by WB and fluorescence. CMA was assessed by lysosomal acquisition of H5C70 and import of cytoplasmic proteins.

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Discussion.—H4B4 is specifically taken up by macrophages and traffics to lysosomes where it reduces membrane LAMP-2 and disrupts function. It markedly inhibits basal CMA and abrogates its stress-induced augmentation. There are clear implications for macrophage function is obvious not least because CMA is critical for antigen presentation to T cells.

Conclusion.—H4B4-triggered CMA dysfunction provides the relevant molecular mechanism for cell death by antibodies to LAMP-2.

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