The lymph self-antigen repertoire

Cristina C. Clement1,2 and Laura Santambrogio1,2 *

1 Department of Pathology, Albert Einstein College of Medicine, New York, NY, USA
2 Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY, USA

Edited by:
Lawrence J. Stern, University of Massachusetts Medical School, USA
Reviewed by:
Philipp Pierre, Centre National de la Recherche Scientifique, France
Josef Mautner, Technische Universität München, Germany
*Correspondence:
Laura Santambrogio, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Forchheimer Building Room 140, Bronx, New York, NY 10461, USA
e-mail: laura.santambrogio@einstein.yu.edu

LYMPH FORMATION

The lymphatic fluid originates from the interstitial fluid which bathes every parenchymal organ and it is generated through a process of ultrafiltration of the plasma, circulating through the blood capillaries, as well as by the addition of metabolic and catabolic products collected from the tissue of origin (1–4).

Once the proteins have been filtered into the extracellular space, they will not re-enter the blood circulatory system by uptake into the venous capillaries as previously thought. Indeed, what was known as the Starling principle has been recently revisited and it is now apparent that almost all the extravasated fluid will be drained into the lymphatics (5).

In addition to the proteins and molecules originating from plasma ultrafiltration, the interstitial fluid will be further enriched with products derived from tissue/organ catabolism/metabolism (6–13).

The interstitial fluid will then drain into open end lymphatic capillaries and hence forth be called lymph (14, 15). The pre-nodal lymph will flow into progressively larger collectors up to the draining lymph nodes (500–600 in humans), disseminated throughout the body. All lymph passes through one or more lymph nodes and each node collects lymph from a distinct region of the body (4). Thus, a molecular signature of tissue-specific self proteins is collected in each node.

LYMPH PROTEOME, DEGRADOME, AND PEPTIDOME

During the last two decades there has been an increasing interest in the analysis of the protein composition of human and rodent lymph under physiological and pathological conditions and in the comparative analysis with plasma samples (6–13, 16–20). This analysis has been elusive for many years due to the difficulty in cannulating lymphatics, which run much deeper than veins and have a smaller diameter and more fragile walls. Additionally, mass spectrometric techniques employed just a few years ago were much less sensitive than they are now in mapping proteins expressed at low levels within scarce amounts of collected fluid.

The lymphatic fluid originates from the interstitial fluid which bathes every parenchymal organ and reflects the “omic” composition of the tissue from which it originates in its physiological or pathological signature. Several recent proteomic analyses have mapped the proteome-degradome and peptidome of this immunologically relevant fluid pointing to the lymph as an important source of tissue-derived self-antigens. A vast array of lymph-circulating peptides have been mapped deriving from a variety of processing pathways including caspases, cathepsins, MMPs, ADAMs, kallikreins, calpains, and granzymes, among others. These self peptides can be directly loaded on circulatory dendritic cells and expand the self-antigenic repertoire available for central and peripheral tolerance.

Proteomic profiles have been reported for human, rodent, bovine, and ovine lymph, and two major conclusions can be drawn from the compilation of these: (6–13, 16–20).

(i) The lymph proteomic profile is not merely overlapping with the one from the plasma but qualitative and quantitative differences can be appreciated; indeed the lymph proteome appears to be enriched in products deriving from tissue and cellular metabolism/catabolism, organ remodeling, extracellular matrix processing, and cellular apoptosis.

(ii) The proteomic molecular signature reflects the tissue from where the lymph is collected and the organ’s physiological or pathological condition. Indeed tissue-specific proteins have been mapped in the lymph collected from capillaries draining specific organs and infectious or inflammatory tissue conditions are reflected in proteomic changes in lymph more so than in plasma.

The proteomic profile of the lymph also revealed the presence of several low molecular weight products composed by fragments, derived from protein processing, and short peptides (12). A similar degradome and peptidome was previously mapped in the plasma and serum and other biological fluids; the most comprehensive analysis so far reports up to 6000 peptides, identified with high confidence in mouse serum (21). Several more groups reported on the low molecular weight cleaved proteome and peptidome revealing the remarkable richness of protein fragments and naturally processed peptides present in lymph, plasma, synovial fluid, urine, and cerebrospinal fluid (22–28). Our group recently mapped the first peptidome transported by the human lymph. Over 300 self peptides were sequenced which derived from the catabolic processing of both intracellular and extracellular proteins (12). The peptidome comprised processed proteins derived from extracellular matrix proteins, cell adhesion molecules, and plasma membrane/receptors as well as an intracellular-derived peptidome consisting of fragments of cytosolic, nuclear (transcription factors
and regulators of gene expression), mitochondrial, endosomal, Golgi, and endoplasmic reticulum proteins (12).

Peptide quantification by \(^{15}N/^{15}N\) labeling and amino acid sequencing from 2D-DIGE spots indicated that many peptides were present in human lymph in at least nanomolar concentrations (12) and analysis of peptide half life in biological fluids indicated a stability of over 24 h (22).

Collectively all the experimental findings point to the lymph as an important biological fluid that transport the tissue "omics" (proteomes, degradomes, peptidomes) to the draining lymph nodes to convey a snapshot of each parenchymal organ in physiological and pathological conditions.

**PROCESSING THE LYMPH AND PLASMA DEGRADOME AND PEPTIDOME**

Two major advances have improved our capability in identifying the lymph and plasma degrade and peptidome's processing pathways; (i) improved mass spectrometric techniques, which allow high confidence peptide identification and correct amino acid assignment and (ii) increased representation of proteins in databases (Brenda, CutD, MEROPS), which facilitated prediction of the processing enzymes involved in peptide cleavage. Analysis of the human lymph- and plasma-carried peptidome identified peptides derived from both intra and extracellular sources and mapped several proteases likely involved in peptide cleavage.

Analyzing the human lymph- and plasma-carried peptidome identified multiple pathways; (i) improved mass spectrometric techniques, which allow high confidence peptide identification and correct amino acid assignment and (ii) increased representation of proteins in databases (Brenda, CutD, MEROPS), which facilitated prediction of the processing enzymes involved in peptide cleavage. Analysis of the human lymph- and plasma-carried peptidome identified peptides derived from both intra and extracellular sources and mapped several proteases likely involved in peptide cleavage.

The lymph and plasma degradome are collected.

Individually and collectively, the lymph and plasma as important biological fluids that transport the tissue "omics" (proteomes, degradomes, peptidomes) to the draining lymph nodes to convey a snapshot of each parenchymal organ in physiological and pathological conditions.

Indeed, in pathological conditions, the mapped peptide/degradome is highly enriched with new peptides as compared to the peptidome/degradome found in healthy physiological conditions (4, 7, 21, 22, 24, 29, 31, 39, 41, 42). This reflects the increased number of peptide fragments cleaved by proteases up-regulated during inflammation (21, 22, 24).

**MHC II LOADING OF THE SELF PEPTIDOME**

MHC II peptide complexes can be loaded in late endosomal MHC-II compartments, early endosomes and at the plasma membrane (43–53). In the late endosomal compartment, antigen processing is dependent on cysteine, aspartic and asparagine endopeptidases, and MHC-II loading depends on the editing molecule HLA-DM (54–57). In early endosomes the antigen processing and MHC II loading is cathespins and mostly HLA-DM-independent (49, 53, 58–60). Similarly extracellular peptides can be loaded at the cell surface either on empty MHC II molecules or through peptide exchange (47, 50, 52, 61–63).

Having distinct MHC class II loading compartments allows presentation of a larger array of peptides. Indeed many of the peptides loaded in recycling/early endosomes and at the cell surface are low affinity and are eliminated by HLA-DM in endosomal compartments (64). Thus, if the MHC endosomal antigen processing and loading machinery restrict the array of presented peptides by kinetic stability and HLA-DM editing, generating an overall higher affinity, higher stability, long-lived MHC-II peptidome (65), the HLA-DM-independent pathway generates a broader, lower stability/easily exchangeable MHC II peptidome.

These two different pathways are exploited by the antigen-presenting cells (APC) to control immunogenicity (65, 66).

Indeed the early endosomes/plasma membrane MHC-II loading pathway is active in immature dendritic cells (DC) and down-regulated upon DC maturation (50–52, 64). As a result immature DC present an overall broader MHC-II peptidome that includes low affinity/stability peptides which, by diluting the high affinity self peptides, contribute to the maintenance of self tolerance. The importance of maintaining a broader MHC-II peptidome under physiological conditions is further supported by the notion that in immature DC and non-stimulated B cells the HLA-DM editing activity is down regulated, within the MHC-II endosomal pathway, by HLA-DO; resulting in decreased presentation of high affinity self peptides that could induce autoimmunity (66, 67). Upon APC maturation/activation surface MHC-II loading is shut off (50–52) and HLA-DM editing activity is up-regulated (66, 67). This would favor presentation of high affinity pathogen-derived peptides to generate immunity.

**MHC II PRESENTATION OF THE SELF PEPTIDOME**

Different mechanisms ensure that tissue-derived self antigens are constantly presented to the immune system for the maintenance of central and peripheral self tolerance (68–70).

In the thymus, medullary epithelial cells (mTEC), conventional dendritic cells (cDC) (SIRPα+ CD11c+ CD8α+), C8α+ DC (SIRPα– CD11c+ CD8α+), and plasmacytoid DC delete immature thymocytes with high affinity for the self MHC-II peptidome (71). These populations of APC display an MHC-II-bound peptidome...
derived from exogenous antigens, acquired through phagocytosis, and endogenous antigen acquired through autophagy (69, 72). Additionally, a subset of mTEC expresses the transcriptional regulator AIRE which promotes expression of tissue-specific antigens, expanding the antigen repertoire to be presented (73). Since mTEC are equipped with all the proteins associated with the antigen processing and presentation machinery, they can directly process the AIRE-expressed antigens. Indeed the presence of autophagosomes in these cells indicates that antigens could enter the endosomal tract through autophagy (72). On the other hand several reports also indicate that mTEC can hand over AIRE-acquired antigens to cDC for thymic selection (74).

Self-antigens in the thymus can also give rise to natural or thymic Tregs, through an avidity-dependent selection process. The APC controlling the formation of Treg are the same as those involved in the process of negative selection (75).

Cells that escape thymic deletion are tolerized in the periphery through anergy or Treg-mediated suppression by tissue and nodal resident dendritic cells (DCs) and macrophages (MΦ) which continuously process and present the self proteome of parenchymal organs. Additionally, AIRE-independent mechanisms have also been described in the periphery which mediate expression of tissue-specific self antigens by lymphatic endothelial cells, further expanding the presentation of the self proteome (76).

All the described mechanisms depend on self-antigen delivery to endosomal compartments, through endocytosis or autophagy. Thus, they generate an MHCII peptidome mostly restricted by endosomal processing enzymes (77).

Recently, circulating cDC have been shown to promote both central and peripheral tolerance by displaying circulating self-antigens to immature thymocytes or mature peripheral T cells (78, 79). Indeed, even though it has been known for some time that intrathymic injection of organ specific APC induce long lasting tolerance to the organ self antigens (80, 81) it was the Goldscheider group that linked thymic tolerance, to extrathymic self antigens, to the role of cDC (82, 83). Work from his group and others indicated that under physiological conditions migratory DC transport self-antigens to mediate thymic negative selection or peripheral T cell anergy and Treg differentiation. In both humans and mice, circulating DC differentiate in a FLT3-dependent manner and are CD11c+; CCR7+; CD103+, and express high levels of MHC II and intermediate expression of co-stimulatory molecules (84). Importantly, migratory DC do not only rely on endosomal processing to display the MHC II peptidome but are capable of loading exogenous peptides as well. Indeed peptides have been shown to induce thymic negative selection not only when directly injected in the thymus (85) but also when injected in the blood stream (78, 86) or in the peritoneum (87–90) which is connected to mesenteric lymphatic drainage (91). Importantly, circulating self antigens have been shown to induce thymic negative selection at physiological concentrations, as the ones achieved on MHC II on the surface of APC (92).

**CONCLUSION**

The interaction between MHC II-peptides and TCRs constitutes the molecular base for all CD4 T cell-mediated immune responses and the displayed MHC II peptidome is critical to the generation of tolerance, immunity, and autoimmunity. The loaded MHC II peptidome is selected based on MHC II affinity, presence or absence of HLA-DM activity, and arrays of available peptides. The degradome/peptidome present in the extracellular milieu and transported by the plasma, lymph, and other biological fluids could contribute to the generation of the MHC II peptidome. Indeed, in the last few years a series of proteomic analysis indicated that the amount of peptides present in biological fluids (lymph, blood, urine, peritoneal fluid) is much higher than what previously known, it is broader in repertoire and has a long half life (4, 21, 22, 24, 29–31, 39). These peptides could function in thymic negative selection similarly to the ones injected exogenously (78, 85–90, 92). Distinct from the peptidome generated in MIIC, the peptide carried by the lymph/plasma is not restricted by endosomal proteases but originates from several other processing pathways, further expanding the self antigen repertoire presented by circulating DC for the maintenance of tolerance.

**ACKNOWLEDGMENTS**

We would like to thank Drs Teresa Di Lorenzo and Moshe Sadofsky for critical reading of this review.

**REFERENCES**

1. Levack JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* (2010) 87:198–210. doi:10.1093/cvr/csp062
2. Rockson SG. The lymphatic cutaneous revisited. *Ann N Y Acad Sci* (2008) 1131:X–X. doi:10.1196/annals.1413.000
3. Interweicz B, Olzewski WL, Leak LV, Petricoin EF, Liotta LA. Profiling of normal human leg lymph proteins using the 2-D electrophoresis and SELDI-TOF mass spectrophotometry approach. *Lymphology* (2004) 37:65–72.
4. Clement CC, Rotzschke O, Santambrogio L. The lymph as a pool of self-antigens. *Trends Immunol* (2011) 32:6–11. doi:10.1016/j.it.2010.10.004
5. Michel CC. *Microvascular Fluid Filtration and Lymph Formation*. New York: Springer Press (2013). p. 35–51.
6. Leak LV, Liotta LA, Krutzsch H, Jones M, Fusaro VS, Ross SJ, et al. Proteomic analysis of lymph.* Proteomics* (2004) 4:753–65. doi:10.1002/pmc.200300573
7. Meng Z, Veenstra TD. Proteomic analysis of serum, plasma, and lymph for the identification of biomarkers. *Proteomics Clin Appl* (2007) 1:747–57. doi:10.1002/prca.200700243
8. Goldfinch GM, Smith WD, Imrie L, McLean K, Inglis NF, Pemberton AD. The proteome of gastric lymph in normal and nematode infected sheep. *Proteomics* (2008) 8:1909–18. doi:10.1002/pmc.200700331
9. Mittal A, Middleitch M, Ruggiero K, Buchanam CM, Jullig M, Loveday B, et al. The proteome of rodent mesenteric lymph. *Am J Physiol Gastrointest Liver Physiol* (2008) 295:G895–903. doi:10.1152/ajpgi.90378.2008
10. Mittal A, Phillips AR, Middleitch M, Ruggiero K, Loveday B, Delahunt B, et al. The proteome of mesenteric lymph during acute pancreatitis and implications for treatment. *JOP* (2009) 10:130–42.
11. Zaravel A, Moore EE, Pelz ED, Jordan JR, Damle S, Dziejatkowska M, et al. Proteomic profiling of the mesenteric lymph after hemorrhagic shock: differential gel electrophoresis and mass spectrometry analysis. *Clin Proteomics* (2010) 8:1–6. doi:10.1186/1559-0275-8-1
12. Clement CC, Cannizo ES, Nastke MD, Sahu R, Olzewski W, Miller NE, et al. An expanded self-antigen peptidome is carried by the human lymph as compared to the plasma. *PLoS One* (2010) e94965. doi:10.1371/journal.pone.0009863
13. Clement CC, Aphkhazava D, Nieves E, Callaway M, Olzewski W, Rotzschke O, et al. Protein expression profiles of human lymph and plasma mapped by 2D-DIGE and 1D SDS-PAGE coupled with nanoLC-ESI-MS/MS bottom-up proteomics. *J Proteomics* (2013) 78:172–87. doi:10.1016/j.jprot.2012.11.013
14. Zawieja DC. Contractile physiology of lymphatics. *Lymphatic Res Biol* (2009) 7:87–96. doi:10.1089/irb.2009.0007
57. Busch R, Rinderknecht CH, Roh S, Lee AW, Harding JJ, Burster T, et al. Achieving stability through editing and chaperoning: regulation of MHC class II peptide binding and expression. *Immunol Rev* (2005) **207**:242–60. doi:10.1111/j.0105-2896.2005.00306.x

58. Kanna R, Burrows SR, Steigerwald-Mullen PM, Moss DJ, Kurilla MG, Cooper L. Targeting Epstein-Barr virus nucleic antigen 1 (EBNA1) through the class II pathway preserves immune recognition by EBNA1-specific cytotoxic T lymphocytes: evidence for HLA-DM-independent processing. *Int Immunol* (1997) **9**:1537–43. doi:10.1093/immunol/9.10.1537

59. Griffin JP, Chu R, Harding CV. Early endosomes and a late endocytic compartment generate different peptide-class II MHC complexes versus distinct processing mechanisms. *J Immunol* (1997) **58**:1523–6.

60. Pu Z, Lovitch SB, Bikoff EK, Unanue ER. T cells distinguish MHC-peptide complexes formed in separate vesicles and edited by H2-DM. *Immunity* (2004) **20**:667–76. doi:10.1016/S1074-7613(04)00073-1

61. Chou CL, Mirshahidi S, Su KW, Kim A, Narayan K, Khoruzhenko S, et al. Short peptide sequences mimic HLA-DM functions. *Mol Immunol* (2008) **45**:1935–43. doi:10.1016/j.molimm.2007.10.033

62. Potalicchio I, Chitta S, Xu X, Fonseca D, Crisi G, Horejsi V, et al. Conformational variation of surface class II MHC proteins during myeloid dendritic cell differentiation accompanies structural changes in lysosomal MHC. *J Immunol* (2005) **175**:4935–47.

63. Eisen HH, Houn XH, Shen C, Wang K, Tantuguri VK, Smith C, et al. Promiscuous binding of extracellular peptides to class I MHC protein. *Proc Natl Acad Sci U S A* (2012) **109**:4580–5. doi:10.1073/pnas.120586109

64. Lovitch SB, Esparraz TJ, Schweitzer G, Herzog J, Unanue ER. Activation of type B T-cells after protein immunization reveals novel pathways of in vivo presentation of peptides. *J Immunol* (2007) **178**:122–33.

65. Lazarsi CA, Chaves FA, Jenks SA, Wu S, Richards KA, Weaver JM, et al. The kinetic stability of MHC class II peptide complexes is a key parameter that dictates immunodominance. *Immunity* (2005) **23**:29–40. doi:10.1016/j.immuni.2005.05.009

66. Yi W, Seth NP, Martillolli T, Wucherpfennig KW, Sant’Angelo DB, Denzin LK. Targeted regulation of self-peptide presentation prevents type I diabetes in mice without disrupting general immunecompetence. *J Clin Invest* (2010) **120**:1324–6. doi:10.1172/JCI40200

67. Fallas JL, Tobin HM, Lou O, Guo D, Sant’Angelo DB, Denzin LK. Ectopic expression of HLA-DO in mouse dendritic cells diminishes MHC class II antigen presentation. *J Immunol* (2004) **173**:1549–60.

68. Kappler JW, Roehm N, Marrack P. T-cell tolerance by clonal elimination in the thymus. *Cell* (1987) **49**:273–80. doi:10.1016/0092-8674(87)90568-X

69. Gallegos AM, Bevan MJ. Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. *J Exp Med* (2004) **200**:1039–49. doi:10.1084/jem.20041457

70. Skokos D, Shakkhar G, Varma R, Waite JC, Cameron TO, Lindquist RL, et al. Peptide-MHC potency governs dynamic interactions between T cells and dendritic cells in lymph nodes. *Nat Immunol* (2007) **8**:835–44. doi:10.1038/ni.1490

71. Mouchless ML, Anderson M. Central tolerance induction. *Curr Top Microbiol Immunol* (2014) **373**:69–86. doi:10.1007/82_2013_321

72. Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L. Autophagy in thymic differentiation accompanies structural changes in lysosomal MIIC. *Cell Immunol* (2014) **299**:123–35. doi:10.1016/j.cellimm.2014.07.008

73. Terzic V, Renshaw DA, Turbock EJ, Fujita A, Wang Z, Nadeau S, et al. Mechanisms of acquired thymic tolerance in experimental autoimmune encephalomyelitis: thymic dendritic-enriched cells induce specific peripheral T-cell unresponsiveness in vivo. *J Exp Med* (1995) **182**:357–66. doi:10.1084/jem.182.2.357

74. Lilblau RS, Tisch R, Shokat K, Yang X, Dumont N, Goodnow CC, et al. Intravenous injection of soluble antigen induces thymic and peripheral T-cell apoptosis. *Proc Natl Acad Sci U S A* (1990) **87**:3031–6. doi:10.1073/pnas.87.7.3031

75. Murphy KM, Heimberger AB, Loh DY. Induction by antigen of intrathymic apoptosis of CD4+CD8+TCRαβ thymocytes in vivo. *Science* (1990) **250**:1720–3. doi:10.1126/science.2125367

76. Mamalaki C, Norton T, Tanaka Y, Townsend AR, Chandler P, Simpson E, et al. Thymic deletion and peripheral activation of class I major histocompatibility complex-restricted T cells by soluble peptide in T-cell receptor transgenic mice. *Proc Natl Acad Sci U S A.* (1992) **99**:11342–6. doi:10.1073/pnas.89.23.11342

77. Zal T, Volkmann A, Stockinger B. Mechanisms of tolerance induction in major histocompatibility complex class II-restricted T cells specific for a blood-borne self-antigen. *J Exp Med* (1994) **180**:2089–99. doi:10.1084/jem.180.6.2089

78. Martin S, Bevan MJ. Antigen-specific and nonspecific deletion of immature cortical thymocytes caused by antigen injection. *Eur J Immunol* (1997) **27**:2726–36. doi:10.1002/eji.1802710370

79. Ashgar RB, Davies SJ. Pathways of fluid transport and reabsorption across the peritoneal membrane. *Kidney Int* (2008) **73**:1048–53. doi:10.1038/ki.2008.32

80. Volkmann A, Zal T, Stockinger B. Antigen-presenting cells in the thymus that can negatively select MHC class II-restricted T cells recognizing a circulating self-antigen. *J Immunol* (1997) **158**:693–706.

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 September 2013; accepted: 20 November 2013; published online: 16 December 2013.

Citation: Clement CC and Santambrogio L (2013) The lymph self-antigen repertoire. *Front. Immunol.* 4:424. doi:10.3389/fimmu.2013.00424

This article was submitted to Antigen Presenting Cell Biology, a section of the journal *Frontiers in Immunology*. Copyright © 2013 Clement and Santambrogio. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.