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Commentary

iBALT and nodular lymphoid hyperplasia in TNF-overexpressing mice

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Abstract

In response to any danger signal, cytokines are promptly secreted to help fight back the attackers. Tumour necrosis factor (TNF) is one of the most potent inflammatory cytokines and its expression is tightly regulated to prevent uncontrolled inflammation. We have shown that three regulatory elements located in *Tnf* 3'untranslated region (3'UTR) cooperate post-transcriptionally to maintain low levels of *Tnf* expression during homeostasis. We have generated several mouse mutants lacking one or two of these regulatory elements. Consequently, these mice overexpress TNF constitutively and develop inflammatory diseases such as rheumatoid arthritis, heart valve disease or inflammatory bowel disease. These mice also present with inducible bronchus-associated lymphoid tissue (iBALT) and nodular lymphoid hyperplasia (NLH) in the bone marrow. We discuss here the role of TNF in the development of tertiary lymphoid organs

Keywords: TNF, SLO, TLO

Abbreviations: APC: Antigen-Presenting Cell; ARE: AU-Rich Element; BALT: Bronchus-Associated Lymphoid Tissue; BM: Bone Marrow; CDE: Constitutive Decay Element; FDC: Follicular Dendritic Cell; GALT: Gut-Associated Lymphoid Tissue; HVD: Heart Valve Disease; IBD: Inflammatory Bowel Disease; NRE: New Regulatory Element; PP: Peyer's Patch; RA: Rheumatoid Arthritis; SLO: Secondary Lymphoid Organ; TLO: Tertiary Lymphoid Organs; TNF: Tumour Necrosis Factor; 3'UTR: 3'Untranslated Region

Introduction

Primary lymphoid organs provide the milieu for the formation and maturation of T cells (thymus) and B cells (bone marrow). Secondary lymphoid organs (SLO) are peripheral lymphoid structures that facilitate the encounter of antigen-presenting cells (APCs) with rare populations of circulating antigen-specific T and B lymphocytes. Once T lymphocytes recognize their complementary APC via their cognate receptors, they cease to migrate, and differentiate into effector and memory cells of identical antigen specificity. On the other hand, B lymphocytes that recognize the same antigen can be activated by its cognate T cell to produce antibodies. Together, they mount a more efficient adaptive immune response.

SLOs include organs such as the spleen, lymph nodes, Peyer's patches and other mucosal-associated lymphoid organs. Since the primary role of SLO is immune surveillance, their development is pre-programmed and occurs already during embryogenesis or early after birth [1]. They are strategically positioned throughout the body to optimally sample antigens coming from the bloodstream and the afferent lymphatics.

Ectopic or tertiary lymphoid organs (TLOs) develop exclusively after birth following antigenic exposure in tissues that are not predisposed to host lymphocytes, such as the lung [2], kidney [3] and liver [4]. Bronchus-associated lymphoid tissue (BALT) is the most studied TLO.

BALT and iBALT

While BALT is normally found around the airways of some mammalian species such as rats and rabbits [5], it is absent from the lungs of healthy mice and humans. Thus, the term "inducible bronchus-associated lymphoid tissue" (iBALT) was coined by Troy Randall to describe the TLO that develops in the lungs of mice and humans following antigenic stimuli, such as exposure to microbes, self-antigens, allergens or even tumors [2,6]. BALT is located near the major bronchi and adjacent to the bronchial epithelium [7], while iBALT can be located throughout the lung [8]. iBALT may appear long after birth in mice and humans as the result of chronic inflammation or infection [6].

TLOs and SLOs have some similarities, as they display segregation into B cell follicles and a T cell zone, interspersed by myeloid cells, stromal cells and follicular dendritic cells. However, unlike SLOs, TLOs usually never acquire the complex structural compartmentalization of SLOs, they are not encapsulated, and the presence of functional lymphatic vessels remains unclear [9-11]. In its simplest form, BALT may only consist in a B cell follicular structure with histologically identifiable FDCs [12].

The development of TLOs such as iBALT results from a complex secretion and signaling of interleukins, chemokines and cytokines (reviewed in Marin et al. [2]). Early signaling via the IL23, IL17 and IL22 is necessary to recruit and organize the myeloid and lymphoid cells in iBALT. These cytokines induce the production of chemokines (CCL19, CCL21, CXCL12, CXCL13) and their receptors (CCR7, CXCR4, CXCR5) which are necessary for the structural organization of iBALT, and to maintain the secretion of lymphotoxins (LT) and TNF needed for stroma priming and differentiation [2,13]. In particular, CXCL13 alone can mediate the homing of lymphocytes to the follicular compartment [2]. While LTα and TNF-α are key signals required for the maintenance of SLOs [14,15], in the context of iBALT they seem to play a role only in some models of infection and chronic inflammation [6,16,17].

TNF Overexpression as an Inducer of iBALT

We have recently described BPSM1 mice, a new spontaneous model of chronic TNF overexpression [18]. Increase in TNF expression in these mice is due to the dysregulation of post-transcriptional regulation caused by the insertion of a retrotransposon in the 3'untranslated region (3'UTR) of the *Tnf* gene. The mutation is dominant, and BPSM1 mice develop rheumatoid arthritis and heart valve disease, but they also present with iBALT and unusual lymphoid nodules in the bone marrow [19]. Surprisingly, iBALT development was the earliest detectable phenotype in BPSM1 mice, appearing about ten days after birth, long before the first signs of arthritis or heart disease. Although we are not specialists of iBALT development or function, we have made, in BPSM1 and subsequent mouse models, a few observations that may be of interest to researchers in this field.

Regulatory Elements in TNF 3'UTR

The role of excessive TNF in many inflammatory diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), inflammatory bowel disease (IBD), psoriasis has been recognized for a long time, and TNF-lowering drugs have been best sellers for several years. Post-transcriptional regulation plays a major role in keeping TNF levels low during homeostasis [20], and we have demonstrated that three regulatory elements in *Tnf* 3'UTR cooperate to efficiently maintain low levels of expression throughout life [21]. The regulatory elements are the AU-rich element (ARE) [20], the constitutive decay element (CDE) [22] and a new regulatory element (NRE) that we have discovered [18]. Figure 1 shows the different genetic mutations

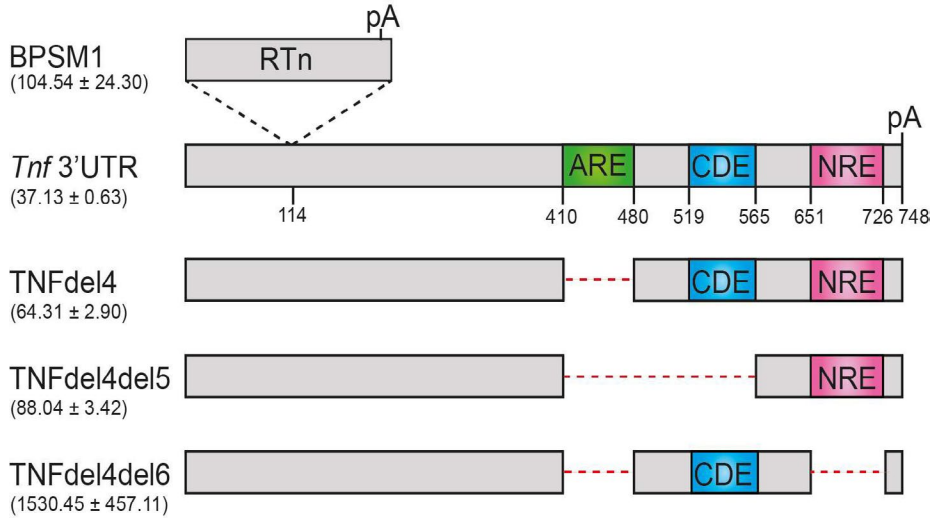

Figure 1: Mutations in *Tnf* 3'UTR cause overexpression of the cytokine. Schematic representation of mouse *Tnf* 3'UTR with numbering relative to the stop codon. BPSM1 mice have a retrotransposon (RTn) inserted at position 114; TNFdel4, TNFdel4del5 and TNFdel4del6 mice have a deletion of one or two of the indicated regulatory elements. Values in parentheses are serum concentrations of TNF (± SEM) in the corresponding mutants.

in Tnf 3'UTR that we have produced during our study of *Tnf* post-transcriptional regulation and have relevance to the development of iBALT. BPSM1, TNFdel4, TNFdel4del5 and TNFdel4del6 mutant mice show various levels of TNF overexpression (Figure 1) that result in phenotypes that differ in severity and tissue localization [18,19,21]. All of them however develop iBALT and/or bone marrow lymphoid nodules. It is important to note that concomitant loss of TNFR1 abrogated all phenotypes in these mice, demonstrating that TNFR2 had no role in any of them.

While numerous triggers including viruses, fungi, bacteria, microbial products and microparticles have been used to induce iBALT formation in mice [2], it is important to note that none of these triggers were used in our various *Tnf* mutants. Natural breathing in itself is obviously not sufficient to induce iBALT in mice in a normal situation. However, it is tempting to speculate that excess TNF conditions the lungs tissue to form iBALT in response to the colonization of the lungs by the microbiota, similar to the necessity of gut microbiota for the formation of isolated lymphoid follicles (ILFs) in the small intestine in mice [23]. A previous study has also shown that pigs, which can spontaneously develop iBALT after birth, never developed them when bred under germ-free conditions [24]. It will thus be interesting to breed our *Tnf* mutants in a germ-free environment to verify this hypothesis.

While BPSM1 mice develop arthritis and heart disease, the primary target of inflammation in homozygous TNFdel4 mice is the gut. Indeed, gut-associated lymphoid tissue (GALT) is hyperplastic in TNFdel4$^{m/m}$ mice: while their Peyer's patches (PPs) appear normal, the lamina propria is invaded by B cells and neutrophils, and numerous ILFs were present, both in the intestine and the colon. Interestingly, ILFs are considered *de novo*-induced TLOs formed in response to luminal stimuli, including normal bacterial flora, and they require TNFR1 function to develop.

TNFdel4del5 heterozygous mice develop arthritis, heart disease, IBD, iBALT and NLH. Due to extremely high levels of serum TNF, all TNFdel4del6 die before birth. However, removal of one TNFR1 allele allows a few heterozygous TNFdel4del6$^{m/+}$/Tnfr1$^{+/-}$ to survive for about 20 days, at which time they present with iBALT in their lungs.

Interestingly, we have never observed lymphoid nodules in the joints or the heart of any of our TNF-overexpressing mice, despite severe arthritis and valve hyperplasia.

B cells are Necessary for iBALT Development, but T cells are Not

B cells are an essential component of BALT and iBALT. Since we wanted to assess the role of B cells in the development of arthritis and heart disease in BPSM1 mice, we crossed them with Mb1-cre mice, in which B cell development is blocked at the pre-B cell stage [25]. While the loss of B cells did not alter the course of arthritis or heart disease, it completely abrogated the development of iBALT in BPSM1 mice. In this particular context, the presence of iBALT does not appear to be pathological.

We also crossed BPSM1 mice with CD3-ε-deficient mice [26] to assess the impact of the loss of T cells on the disease. Once again, the development of arthritis and heart disease was not affected by the absence of T cells, nor was the development of iBALT. This was rather surprising since IL-17 produced by CD4$^+$ T cells had been suggested to be essential for the formation of iBALT [27]. When we crossed BPSM1 to IL-17-deficient mice, we observed that iBALT still developed in double mutant mice. Infection with Modified Vaccinia virus Ankara could also induce iBALT formation in IL-17-deficient mice [28]. Thus, the actual importance of IL-17 in iBALT development remains unclear.

Since pulmonary administration of LPS is commonly used to trigger iBALT formation in mice, it is interesting to note that ablation of Myd88 in BPSM1/Myd88 double mutant mice did not prevent the formation of iBALT.

Bone marrow lymphoid nodules: a novel TLO?

While iBALT has been the focus of much research, the scientific literature contains few reports on bone marrow lymphoid nodules, even though they have been known to exist since 1915 (see Rywlin et al. [29]). They have been described in patients suffering from a variety of infectious or inflammatory diseases, as well as in patients with chronic lymphocytic leukaemia. Although benign lymphoid follicles appear to be not so rare in human patients [29-31], most of the studies that have reported them were made on samples obtained from older patients or patients with some kind of disease and thus do not accurately represent the wider population. In most cases, the presence of BM follicles seems to be associated with high circulating TNF levels, even in the case of chronic lymphocytic leukaemia. The paucity of data may be related to the difficulty of obtaining bone marrow samples.

NLH is such a prominent feature in our TNF-overexpressing mice that it is hard to comprehend why it has not been reported earlier in other mouse models. At first glance, NLH presents a lot of similarities with iBALT, consisting primarily of B cells and follicular dendritic cells. Like with iBALT, loss of B cells prevented the development of NLH, while the loss of T cells did not. None of our mice ever developed a lymphoid malignancy, leaving no doubt that NLH in our mice is not a premalignant B cell stage. To test this further, we crossed BPSM1 mice with the Eμ-Myc mice [32]. The presence of the BPSM1 mutation did not accelerate lymphomagenesis in the Eμ-Myc mice (E. Clayer and P. Bouillet, unpublished observation). While it may seem strange that a TLO would form within a primary lymphoid organ such as the bone marrow, the parallel development of iBALT and NLH in TNF-overexpressing mice suggests that NLH may in fact be a previously unrecognised TLO.

Hematopoietic Reconstitution Experiments

To better understand the role of the hematopoietic cells in the pathologies of our *Tnf* mutants, we performed reconstitution experiments in lethally-irradiated recipients. The results of these experiments have been described in detail in Seillet et al. [19] and Clayer et al. [21]. The interesting lessons from these experiments were that i) injection of TNF-overexpressing BM cells failed to induce iBALT in most recipients although the same recipients readily developed NLH; ii) TNFR1-deficient recipients developed neither iBALT nor NLH; iii) TNF-overexpressing TNFR1-deficient BM cells induced NLH efficiently in WT recipients, even though the donors of these cells did not have NLH themselves; iv) *BPSM1$^{m/+}$*-irradiated recipients maintained their iBALT, but lost their NLH when transplanted with wild-type (WT) BM cells. These results demonstrated that iBALT and NLH formation is the result of the interaction of hematopoietic cells and TNFR1-sufficient non-hematopoietic cells from the recipients.

iBALT and NLH: Friends or Foes?

Much has been said about the beneficial and deleterious effects associated with the presence of TLOs [2,13,33,34]. The presence of TLOs in some animals in the absence of any inflammation certainly suggests that they are not pathological *per se*, and in many cases they were shown to function as any SLO. In the case of TNF-induced arthritis and valve disease, which are B and T cell-independent, we have shown that iBALT and NLH had no influence on the severity of the disease, and the presence of iBALT helped clear Mycobacteria tuberculosis (Mtb) in BPSM1 mice. However, in the case of autoimmune conditions involving B cells such as rheumatoid factor-positive arthritis, we believe that the increase in B cell numbers due to iBALT and NLH would be an aggravating factor. In fact, it seems reasonable to predict that the nature of the B cells that join the newly formed TLO determines whether it will have a beneficial or pathological effect, simply by increasing their total number.

Conclusion

A lot remains to be done to understand the signals that lead to the formation of TLOs. Although TNF has rarely been considered a primary inducer of these structures, our results suggest otherwise. Our mice represent unique models to explore new hypotheses, in particular the exploration of NLH formation and its possible identity as a TLO. We will be happy to make them available to all experts interested in undertaking such studies.

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References

1. Randall TD, Carragher DM, Rangel-Moreno J. Development of secondary lymphoid organs. Annu Rev Immunol. 2008 Apr 23;26:627-50.
2. Marin ND, Dunlap MD, Kaushal D, Khader SA. Friend or foe: the protective and pathological roles of inducible Bronchus-Associated lymphoid tissue in pulmonary diseases. The Journal of Immunology. 2019 May 1;202(9):2519-26.
3. Pei G, Zeng R, Han M, Liao P, Zhou X, Li Y, et al. Renal interstitial infiltration and tertiary lymphoid organ neogenesis in IgA nephropathy. Clinical Journal of the American Society of Nephrology. 2014 Feb 7;9(2):255-64.
4. Shomer NH, Fox JG, Juedes AE, Ruddle NH. Helicobacter-induced chronic active lymphoid aggregates have characteristics of tertiary lymphoid tissue. Infection and Immunity. 2003 Jun 1;71(6):3572-7.
5. Sminia T, Van der Brugge-Gamelkoorn GJ, Jeurissen SH. Structure and function of bronchus-associated lymphoid tissue (BALT). Critical Reviews in Immunology. 1989;9(2):119.
6. Moyron-Quiroz JE, Rangel-Moreno J, Kusser K, Hartson L, Sprague F, Goodrich S, et al. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. Nature Medicine. 2004 Sep;10(9):927-34.
7. Gregson RL, Davey MJ, Prentice DE. Postnatal development of bronchus-associated lymphoid tissue (BALT) in the rat, Rattus norvegicus. Laboratory Animals. 1979 Jul;13(3):231-8.
8. Moyron-Quiroz JE, Rangel-Moreno J, Hartson L, Kusser K, Tighe MP, Klonowski KD, et al. Persistence and responsiveness of immunologic memory in the absence of secondary lymphoid organs. Immunity. 2006 Oct 1;25(4):643-54.
9. Ruddle NH. Lymphatic vessels and tertiary lymphoid organs. The Journal of clinical investigation. 2014 Mar 3;124(3):953-9.
10. Buckley CD, Barone F, Nayar S, Benezech C, Caamano J. Stromal cells in chronic inflammation and tertiary lymphoid organ formation. Annual Review of Immunology. 2015 Mar 21;33:715-45.
11. Pipi E, Nayar S, Gardner DH, Colafrancesco S, Smith C, Barone F. Tertiary lymphoid structures: autoimmunity goes local. Frontiers in Immunology. 2018 Sep 12;9:1952.
12. Pabst R. Plasticity and heterogeneity of lymphoid organs: what are the criteria to call a lymphoid organ primary, secondary or tertiary?. Immunology Letters. 2007 Sep 15;112(1):1-8.
13. Hwang JY, Randall TD, Silva-Sanchez A. Inducible bronchus-associated lymphoid tissue: taming inflammation in the lung. Frontiers in Immunology. 2016 Jun 30;7:258.
14. Ruddle NH, Akirav EM. Secondary lymphoid organs: responding to genetic and environmental cues in ontogeny and the immune response. The Journal of Immunology. 2009 Aug 15;183(4):2205-12.
15. De Togni P, Goellner J, Ruddle NH, Streeter PR, Fick A, Mariathasan S, et al. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science. 1994 Apr 29;264(5159):703-7.
16. Kuroda E, Ozasa K, Temizoz B, Ohata K, Koo CX, Kanuma T, et al. Inhaled fine particles induce alveolar macrophage death and interleukin-1α release to promote inducible bronchus-associated lymphoid tissue formation. Immunity. 2016 Dec 20;45(6):1299-310.
17. Eddens T, Elsegeiny W, de la Luz Garcia-Hernadez M, Castillo P, Trevejo-Nunez G, Serody K, et al. Pneumocystis-driven inducible bronchus-associated lymphoid tissue formation requires Th2 and Th17 immunity. Cell reports. 2017 Mar 28;18(13):3078-90.
18. Lacey D, Hickey P, Arhatari BD, O'Reilly LA, Rohrbeck L, Kiriazis H, Du XJ, Bouillet P. Spontaneous retrotransposon insertion into TNF 3′ UTR causes heart valve disease and chronic polyarthritis. Proceedings of the National Academy of Sciences. 2015 Aug 4;112(31):9698-703.
19. Seillet C, Carr E, Lacey D, Stutz MD, Pellegrini M, Whitehead L, et al. Constitutive overexpression of TNF in BPSM1 mice causes iBALT and bone marrow nodular lymphocytic hyperplasia. Immunology and Cell Biology. 2019 Jan;97(1):29-38.
20. Han J, Beutler B. The essential role of the UA-rich sequence in endotoxin-induced cachectin/TNF synthesis. European Cytokine Network. 1990;1(2):71.
21. Bouillet P, Clayer E, Dalseno D, Kueh AJ, Lacey D, Tsai M, et al. Severe dysregulation of TNF expression leads to multiple inflammatory diseases and embryonic death. BioRxiv. 2020 Jan 1.
22. Stoecklin G, Lu M, Rattenbacher B, Moroni C. A constitutive decay element promotes tumor necrosis factor alpha mRNA degradation via an AU-rich element-independent pathway. Molecular and Cellular Biology. 2003 May 15;23(10):3506-15.
23. Lorenz RG, Chaplin DD, McDonald KG, McDonough JS, Newberry RD. Isolated lymphoid follicle formation is inducible and dependent upon lymphotoxin-sufficient B lymphocytes, lymphotoxin β receptor, and TNF receptor I function. The Journal of Immunology. 2003 Jun 1;170(11):5475-82.

24. Pabst R, Gehrke I. Is the bronchus-associated lymphoid tissue (BALT) an integral structure of the lung in normal mammals, including humans?. American Journal of Respiratory Cell and Molecular Biology. 2012 Dec 14.

25. Hobeika E, Thiemann S, Storch B, Jumaa H, Nielsen PJ, Pelanda R, et al. Testing gene function early in the B cell lineage in mb1-cre mice. Proceedings of the National Academy of Sciences. 2006 Sep 12;103(37):13789-94.

26. Malissen M, Gillet A, Rocha B, Trucy J, Vivier E, Boyer C, et al. T cell development in mice lacking the CD3-zeta/eta gene. The EMBO Journal. 1993 Nov;12(11):4347-55.

27. Rangel-Moreno J, Carragher DM, de la Luz Garcia-Hernandez M, Hwang JY, Kusser K, Hartson L, et al. The development of inducible bronchus-associated lymphoid tissue depends on IL-17. Nature Immunology. 2011 Jul;12(7):639-46.

28. Fleige H, Haas JD, Stahl FR, Willenzon S, Prinz I, Förster R. Induction of BALT in the absence of IL-17. Nature Immunology. 2012 Jan;13(1):1.

29. Rywlin AM, Ortega RS, Dominguez CJ. Lymphoid nodules of bone marrow: normal and abnormal. Blood. 1974 Mar;43(3):389-400.

30. Thiele J, Zirbes TK, Kvasnicka HM, Fischer R. Focal lymphoid aggregates (nodules) in bone marrow biopsies: differentiation between benign hyperplasia and malignant lymphoma--a practical guideline. Journal of Clinical Pathology. 1999 Apr 1;52(4):294-300.

31. Johnston A, Brynes RK, Naemi K, Reisian N, Bhansali D, Zhao X, et al. Differentiating benign from malignant bone marrow B-cell lymphoid aggregates: a statistical analysis of distinguishing features. Archives of Pathology and Laboratory Medicine. 2015 Feb;139(2):233-40.

32. Adams JM, Harris AW, Pinkert CA, Corcoran LM, Alexander WS, Cory S, et al. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. Nature. 1985 Dec 12;318(6046):533-8.

33. Foo SY, Phipps S. Regulation of inducible BALT formation and contribution to immunity and pathology. Mucosal Immunology. 2010 Nov;3(6):537-44.

34. Randall TD. Bronchus-associated lymphoid tissue (BALT): structure and function. In: Advances in Immunology 2010 Jan 1 (Vol. 107, pp. 187-241). Academic Press.