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Brief review

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Anti-apoptosis function of TNF-α in chronic lymphocytic leukemia: lessons from Crohn's disease and the therapeutic potential of bupropion to lower TNF-α

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Summary

Crohn's disease and B cell chronic lymphocytic leukemia (CLL) share a common link in their pathologic mechanisms. Lymphocytes in both diseases fail to undergo apoptosis and die properly. That failure is partly due to increased signaling by tumor necrosis factor (TNF)-α, and their respective pathologies directly follow from this apoptosis failure. Bupropion is a commonly used generic antidepressant in clinical use for over a decade, and early evidence indicates it lowers TNF levels. This paper suggests the use of bupropion in CLL to lower TNF levels, which may thereby slow CLL disease course.

Key words: **apoptosis • bupropion • chronic lymphocytic leukemia • Crohn's disease • etanercept • inflammation • infliximab • lymphocyte • treatment • TNF-α**

Abbreviations: **CLL** – B cell chronic lymphocytic leukemia, **cAMP** – cyclic adenosine monophosphate, **CD** – Crohn's disease, **TNF** – tumor necrosis factor α, **NF-κB** – nuclear factor kappa B.

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INTRODUCTION

This short paper has a simple story to tell: Crohn's disease (CD) and B cell chronic lymphocytic leukemia (CLL) share a common link in their pathologic mechanisms. Lymphocytes in both diseases fail to undergo apoptosis and die properly. That failure is partly due to increased anti-apoptosis signaling by tumor necrosis factor (TNF)-α. The core feature of the two pathologies, though they are clinically quite different, directly follows from this TNF-mediated apoptosis failure. Bupropion is a commonly used generic antidepressant in worldwide clinical use in the treatment of depression for over a decade, and early evidence indicates it may lower TNF levels. Bupropion, in initial clinical use, seems to reliably lower CD activity. This paper suggests the use of bupropion in CLL to lower TNF levels and, thereby, possibly slow CLL disease course.

TNF

TNF is a circulating 17 kDa signaling molecule synthesized by a wide range of cells, reviewed in references[18, 31]. It is also found fixed within the outer cell membrane in a 26 kDa transmembrane form. Both forms are active, potent signaling molecules. Some functions overlap (can be performed by both forms), while some are preferentially performed by one or the other form. All soluble 17 kDa TNF derives from proteolytic cleavage of the transmembrane form. TNF is thought to act only by ligation to one of the two known specific receptors, R1 or R2. TNF effects are supremely pleiotropic, which is essential to the function of many systems. Although considerably more complex than this, TNF function can be roughly categorized as apoptosis triggering (frequently, but not exclusively, through R1) and anti-apoptosis, pro-inflammatory signaling (frequently, but not exclusively, through R2)[18, 31]. Physiologically, these two functions can be and often are simultaneously initiated by TNF, although apoptotic or anti-apoptotic response bias is often seen.

TNF IN CD

Recent advances in our understanding of how the superficial erosions of the gut wall mucosa (aphthae) occur in CD have given clues to a better treatment of some cancers. The documented apoptosis-inhibiting effects of TNF in the lamina propria lymphocytes contribute strongly to the aphthae generation in CD[7, 10, 29]. The relevant lymphocytes do not die off properly and, by persisting, continue generating inflammatory signals that eventually erode the mucosa. Lowering TNF function with the monoclonal anti-TNF antibody infliximab lowers CD activity (ref.[25], and many others), and this has been shown to occur by increasing intramural lymphocyte apoptosis[7, 11, 29]. In initial, uncontrolled clinical use, bupropion seems to be an effective treatment of CD[13, 14, 15].

This paper presents the argument that CLL might be ameliorated by bupropion in a parallel fashion although, we expect, less thoroughly than CD because there are many other derangements in CLL beyond TNF dysregulation. CD is a simpler disease than CLL.

Etanercept is a soluble, TNF R2-based protein for use in the treatment of rheumatoid arthritis. Infliximab is effective in the treatment of CD[25], but etanercept has no activity in CD amelioration[25, 29], which corresponds to observations that infliximab, but not etanercept, induces apoptosis in lamina propria lymphocytes from patients with CD[7, 29].

An essential link in the development of overt CD is created when TNF becomes a survival signal for lamina propria lymphocytes to a pathological degree, this paper suggesting by a manner and mechanism as that in CLL. A TNFR1-to-TNFR2 switch of predominance on the lamina propria lymphocyte surface is seen in CD[10, 11] corresponding to a switch from TNF-induced apoptosis (TNFR1 prominent) to a TNFR2 (pro-survival, anti-apoptotic, nuclear factor (NF)-κB-activating) response.

Why does etanercept not help in CD? We suggest that by further shifting the signaling weight to TNFR2 through a relatively selective inhibition of sTNF and a relatively weak or non-inhibition of tmTNF signaling, the core problem is not redressed. Bupropion may down-regulate TNF synthesis generally, providing a balanced TNF-signaling reduction, without shifting the TNFR1/TNFR2 relative weighting. If this proves to be correct, TNF survival signaling to the problematic persisting lamina propria lymphocytes would be reduced, allowing them to die off and not perpetuate the mutually reinforcing cycle of mucosa breakdown outlined below.

The monoclonal antibody infliximab, by binding more to tmTNF than etanercept does[2, 7, 29], can prevent TNF anti-apoptosis signaling via TNFR2, thus facilitating apoptosis in the problematic lamina propria lymphocytes in CD.

Lamina propria lymphocytes have many housekeeping functions, one of which is defense-early-warning signaling of antigenic attack (predominantly microbial). In CD these lymphocytes mediate a protracted immune/inflammatory response unwarranted by

commensurate luminal aggression[7, 10, 11, 29]. The resulting overly exuberant response damages otherwise healthy and well-functioning nearby mucosa. Mucosal damage provides a protective barrier breech that is taken advantage of by lumen bacteria and viruses; also food antigens gain access to the gut wall substance. This provides additional antigenic stimuli, correctly perceived by lamina propria lymphocytes. The resulting immune response extends mucosal damage. Thus the aphthous lesion of Crohn's is built up by a mutually reinforcing feedback cycle between luminal aggression breech of the gut mucosal barrier and the over-exuberant response to it mediated by anti-apoptotic TNF signaling on lamina propria lymphocytes.

TNF AND CLL

CLL is a prime example of an indolent cancer characterized by abnormally low apoptosis rather than increased mitosis[16, 19]. Although multiple apoptosis-inhibiting factors other than TNF have been identified in CLL (examples in ref.[16, 19]), much data indicate that excess TNF function in the bone marrow is a central mediator of the malignant lymphocytes' apoptosis resistance in CLL:

General

All CLL patients were seen to have PHA-stimulated peripheral blood culture TNF levels greater than controls' [3]. Circulating TNF levels in CLL patients are higher than normal[1, 5, 8] and levels increase with advancing disease stage[1, 8]. Higher TNF levels correlate with shorter survival[8]. Aggressive CLL shows higher circulating TNF levels than do less aggressive forms[23].

TNF and B cells

A greater percentage of circulating and bone marrow B lymphocytes immunostain positive for TNF than normal ones[4]. The malignant B cells themselves synthesize increased TNF *in vivo*[17].

TNF and T cells

Increased T lymphocyte synthesis occurs in peripheral T cells late in CLL upon *in vitro* stimulation[9, 23]. $CD2^+$ lymphocytes (comprising NK cells and 90% of circulating T cells) from CLL patients had higher *in vitro* stimulated TNF synthesis than did normals[20]. T cells show upregulated, increased TNF in the bone marrow of CLL[4, 17] and circulating T cells show upregulated TNF synthesis in CLL[4]. Again, TNF-positive circulating T cells are more numerous in advanced stages of CLL compared with earlier stages[4].

TNF and NF-κB

As has been noted for other B lymphocyte malignancies, for example multiple myeloma, the malignant cells of CLL show chronically and tonically activated NF-κB[21]. *In vitro* apoptosis of CLL cells is invariably preceded by NF-κB activation loss[6]. The activation and transcription of multiple anti-apoptosis genes consequent to NF-κB activation is seen in CLL[34], and TNF is one of the primary activators of NF-κB[18, 31, 34].

TNF receptors

The malignant lymphocytes in CLL bear TNF receptors that are heavily weighted or completely restricted to R2[27, 28, 32]. Note the parallel shift to R2 seen in lamina propria lymphocytes in CD[10, 11] mentioned above.

CONCLUSIONS

Thus the malignant B lymphocytes of CLL are exposed to an abnormally TNF-enriched environment in CLL. One of the curiosities, which is simultaneously a huge hint into its pathogenic mechanism, is the finding of above-normal *in vitro* apoptosis of CLL lymphocytes yet abnormally low *in vivo* apoptosis[12]. It is likely that precisely this TNF-enriched bone marrow environment is what accounts for the reduced *in vivo* apoptosis, and deprivation of that TNF-enriched environment is what allows apoptosis to proceed.

A recently completed phase II trial of theophylline in CLL has shown evidence of a weak but clear activity in slowing disease progress[33]. Theophylline, as a weak phosphodiesterase inhibitor, increases intracellular cyclic adenosine monophosphate (cAMP), thus lowering TNF synthesis. This work shows proof-of-principle. Other more specific phosphodiesterase inhibitors (of the IV isoform), such as rolipram, have shown *in vitro* pre-clinical apoptosis induction in lymphocytes from CLL[26].

Already in 1993, TNF was recognized as a significant growth factor in CLL and a suggested target for therapeutic inhibition[30]. Even then the inverse relationship between intracellular cAMP and TNF was recognized, as was *in vitro* CLL cell proliferation inhibited through increased cAMP reduction of TNF[30].

CAVEAT

Also of concern is the potential for negative interaction between bupropion or any other TNF synthesis-lowering maneuver, for example by thalidomide,

during rituximab treatment. Rituximab is a monoclonal antibody to CD20, a surface antigen found on the malignant lymphocytes in some CLL cases. CD20 antibody treatment of CLL has shown some benefit[22]. There is evidence that lymphocyte CD20 expression can be upregulated by TNF and down-regulated in the absence of sufficient TNF[24]. Therefore treatment with rituximab should be temporally separated from TNF suppression with bupropion. These same cautionary concerns should also apply to the radioactive anti-CD20 treatments Bexxar and Zevalin. Speculatively, one could even consider systemic treatment with TNF-elevating agents[14] prior to and during treatment with these CD20-targeted treatments, followed by anti-TNF treatments, for example with bupropion.

If our currently ongoing clinical success with bupropion treatment of CD holds up in double-blind placebo-controlled trials, we think bupropion might prove to be considerably more potent in TNF synthesis suppression than theophylline, and would therefore be expected to be proportionately more active in CLL disease retardation.

REFERENCES

1. Adami F., Guarini A., Pini M., Siviero F., Sancetta R., Massaia M., Trentin L., Foa R. and Semenzato G. (1994): Serum levels of tumour necrosis factor-alpha in patients with B-cell chronic lymphocytic leukaemia. Eur. J. Cancer, 30A, 1259–1263.
2. Agnholt J., Dahlerup J. F. and Kaltoft K. (2003): The effect of etanercept and infliximab on the production of tumour necrosis factor alpha, interferon-gamma, and GM-CSF in *in vivo* activated T lymphocyte cultures. Cytokine, 23, 76–85.
3. Barcellini W., Montesano R., Clerici G., Zaninoni A., Imperiali F. G., Calori R., Cortelezzi A. and Zanella A. (2002): *In vitro* production of anti-RBC antibodies and cytokines in chronic lymphocytic leukemia. Am. J. Hematol., 71, 177–183.
4. Bojarska-Junak A., Rolinski J., Wasik-Szczepaneko E., Kaluzny Z. and Dmoszynska A. (2002): Intracellular tumor necrosis factor production by T and B cells in chronic lymphocytic leukemia. Haematologica, 87, 490–499.
5. Capalbo S., Battista C., Delia M., Ciancio A., De Santis G., Dargenio M., Diomede D. and Liso V. (2002): Evaluation of tumor necrosis factor-alpha and erythropoietin serum levels in B-cell chronic lymphocytic leukemia patients with anemia. Acta Haematol., 108, 84–89.
6. Cuni S., Perez-Aciego P., Perez-Chacon G., Vargas J. A., Sanchez A., Martin-Saavedra F. M., Ballester S., Garcia-Marco J., Jorda J. and Durantez A. (2004): A sustained activation of PI3K/NF--kappaB pathway is critical for the survival of chronic lymphocytic leukemia B cells. Leukemia, 18, 1391–1400.
7. Di Sabatino A., Ciccocioppo R., Cinque B., Millimaggi D., Morera R., Ricevuti L., Cifone M. G. and Corazza G. R. (2004): Defective mucosal T cell death is sustainably reverted by infliximab in a caspase dependent pathway in Crohn's disease. Gut, 53, 70–77.
8. Ferrajoli A., Keating M. J., Manshouri T., Giles F. J., Dey A., Estrov Z., Koller C. A., Kurzrock R., Thomas D. A., Faderl S., Lerner S., O'Brien S. and Albitar M. (2002): The clinical significance of tumor necrosis factor-alpha plasma level in patients having chronic lymphocytic leukemia. Blood, 100, 1215–1219.
9. Gallego A., Vargas J. A., Castejon R., Citores M. J., Romero Y., Millan I. and Durantez A. (2003): Production of intracellular IL-2, TNF-α, and IFN-γ by T cells in B-CLL. Cytometry B Clin. Cytom., 56, 23–29.
10. Holtmann M. H., Douni E., Schutz M., Zeller G., Mudter J., Lehr H. A., Gerspach J., Scheurich P., Galle P. R., Kollias G. and Neurath M. F. (2002): Tumor necrosis factor-receptor 2 is up-regulated on lamina propria T cells in Crohn's disease and promoters experimental colitis *in vivo*. Eur. J. Immunol., 32, 3142–3151.
11. Holtmann M. H., Schutz M., Galle P. R., and Neurath M. F. (2002): Functional relevance of soluble TNF-α, transmembrane TNF-α and TNF-signal transduction in gastrointestinal diseases with special reference to inflammatory bowel diseases. Z. Gastroenterol., 40, 587–600.
12. Jewell A. P. (2002): Role of apoptosis in the pathogenesis of B-cell chronic lymphocytic leukaemia. Br. J. Biomed. Sci., 59, 235–238.
13. Kane S., Altschuler E. L. and Kast R. E. (2003): Crohn's disease remission on bupropion. Gastroenterology, 125, 1290.
14. Kast R. E. (2003): Anti- and pro-inflammatory considerations in antidepressant use during medical illness: bupropion lowers and mirtazapine increases circulating tumor necrosis factor-α levels. Gen. Hosp. Psychiatry, 25, 495–496.
15. Kast R. E. and Altschuler E. L. (2001): Remission of Crohn's disease on bupropion. Gastroenterology, 121, 1260–1261.
16. Kolb J. P., Kern C., Quiney C., Roman V. and Billard C. (2003): Re-establishment of a normal apoptotic process as a therapeutic approach in B-CLL. Curr. Drug Targets Cardiovasc. Haematol. Disord., 3, 261–286.
17. Mainou-Fowler T., Miller S., Proctor S. J. and Dickinson A. M. (2001): The levels of TNF alpha, IL4 and IL10 production by T--cells in B-cell chronic lymphocytic leukaemia (B-CLL). Leuk. Res., 25, 157–163.
18. Nanes M. S. (2003): Tumor necrosis factor-α: molecular and cellular mechanisms in skeletal pathology. Gene, 321, 1–15.
19. Podhorecka M., Dmoszynska A. and Rolinski J. (2004): Intracellular IFN-γ expression by CD3+/CD8+ cell subset in B-CLL patients correlates with stage of the disease. Eur. J. Haematol., 73, 29–35.
20. Reyes E., Prieto A., Carrion F., Garcia-Suarez J., Esquivel F., Guillen C. and Alvarez-Mon M. (1998): Altered pattern of cytokine production by peripheral blood CD2+ cells from B chronic lymphocytic leukemia patients. Am. J. Hematol., 57, 93–100.
21. Ringshausen I., Dechow T., Schneller F., Weick K., Oelsner M., Peschel C. and Decker T. (2004): Constitutive activation of the MAPkinase p38 is critical for MMP-9 production and survival of B-CLL cells on bone marrow stromal cells. Leukemia, 18, 1964–1970.
22. Robak T., Smolewski P., Urbanska-Rys H., Gora-Tybor J., Blonski J. Z. and Kasznicki M. (2004): Rituximab followed by cladribine in the treatment of heavily pretreated patients with indolent lymphoid malignancies. Leuk. Lymphoma, 45, 937–944.
23. Rossmann E. D., Lewin N., Jeddi-Tehrani M., Osterborg A. and Mellstedt H. (2002): Intracellular T cell cytokines in patients with B cell chronic lymphocytic leukaemia (B-CLL). Eur. J. Haematol., 68, 299–306.
24. Sivaraman S., Deshpande C. G., Ranganathan R., Huang X., Jajeh A., O'Brien T., Huang R. W., Gregory S. A., Venugopal P. and Preisler H. D. (2000): Tumor necrosis factor modulates CD 20 expression on cells from chronic lymphocytic leukemia: a new role for TNF-α? Microsc. Res. Tech., 50, 251–257.
25. Tilg H. and Kaser A. (2002): Antitumour necrosis factor therapy in Crohn's disease. Expert Opin. Biol. Ther., 2, 715–721.
26. Tiwari S., Felekkis K., Moon E. Y., Flies A., Sherr D. H. and Lerner A. (2004): Among circulating hematopoietic cells, B-CLL uniquely expresses functional EPAC1, but EPAC1-mediated Rap1 activation does not account for PDE4 inhibitor-induced apoptosis. Blood, 103, 2661–2667.

27. Trentin L., Zambello R., Agostini C., Enthammer C., Cerutti A., Adami F., Zamboni S. and Semenzato G. (1994): Expression and regulation of tumor necrosis factor, interleukin-2, and hematopoietic growth factor receptors in B-cell chronic lymphocytic leukemia. Blood, 84, 4249–4256.

28. Trentin L., Zambello R., Agostini C., Siviero F., Adami F., Marcolongo R., Raimondi R., Chisesi T., Pizzolo G. and Semenzato G. (1993): Expression and functional role of tumor necrosis factor receptors on leukemic cells from patients with type B chronic lymphoproliferative disorders. Blood, 81, 752–758.

29. Van den Brande J. M., Braat H., van den Brink G. R., Versteeg H. H., Bauer C. A., Hoedemaeker I., van Montfrans C., Hommes D. W,, Peppelenbosch M. P. and van Deventer S. J. (2003): Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. Gastroenterology, 124, 1774–1785.

30. Van Kooten C., Rensink I., Aarden L. and van Oers R. (1993): Cytokines and intracellular signals involved in the regulation of B-CLL proliferation. Leuk. Lymphoma, 12, 27–33.

31. Varfolomeev E. E. and Ashkenazi A. (2004): Tumor necrosis factor: an apoptosis JuNKie? Cell, 116, 491–497.

32. Waage A. and Espevik T. (1994): TNF receptors in chronic lymphocytic leukemia. Leuk. Lymphoma, 13, 41–46.

33. Wiernik P. H., Paietta E., Goloubeva O., Lee S. J., Makower D., Bennett J. M., Wade J. L., Ghosh C., Kaminer L. S., Pizzolo J., Tallman M. S. and Eastern Cooperative Oncology Group (2004): Phase II study of theophylline in chronic lymphocytic leukemia: a study of the Eastern Cooperative Oncology Group (E4998). Leukemia, 18, 1605–1610.

34. Zaninoni A., Imperiali F. G., Pasquini C., Zanella A. and Barcellini W. (2003): Cytokine modulation of nuclear factor--kappaB activity in B-chronic lymphocytic leukemia. Exp. Hematol., 31, 185–190.