Title
GABA molecules made by B cells can dampen antitumour responses

Journal
Nature, 599(7885)

ISSN
0028-0836

Author
Kaufman, Daniel L

Publication Date
2021-11-18

DOI
10.1038/d41586-021-02953-1

Peer reviewed

https://doi.org/10.1038/d41586-021-02953-1

News & views

Immunology

A neurotransmitter limits antitumour responses

Daniel L. Kaufman

Analysis of immune cells shows that, unexpectedly, B cells secrete GABA, a molecule best known as a neurotransmitter. B-cell-derived GABA can modulate immune responses against tumours, raising the prospect of new therapies.

Efforts to better understand how immune cells function hold the promise of providing information that might lead to improved clinical treatments. Writing in *Nature*, Zhang *et al.*[1] present results that point the way to the development of new approaches to enhance anticancer therapies.

The authors investigated changes in metabolite molecules that occurred in mouse lymph nodes — a tissue rich in immune cells — after the animals had been exposed to a foreign protein through immunization. Using state-of-the-art technologies, Zhang and colleagues compared the metabolites in lymph nodes near the immunization site with those in lymph nodes on the opposite side of the animal's body. They found that levels of around 200 metabolites were significantly different in lymph nodes near the immunization site, particularly metabolites associated with activation of a system called the glutamate pathway.

Zhang and colleagues repeated this experiment using mice deficient in immune cells called B cells and T cells, and, by comparing these animals with those not lacking immune cells, found that the predominant metabolic changes after immunization occurred in B cells (which are antibody-producing cells). Surprisingly, the major metabolite upregulated in response to immunization was γ-aminobutyric acid (GABA), which was not previously known to be made by B cells. GABA acts as a neurotransmitter in the brain, with key roles in neurodevelopment. It is linked to certain neurological disorders[2], and is produced through the glutamate pathway.

To explore GABA synthesis in immune cells, the authors investigated B cells and T cells from mice and humans. They activated the cells *in vitro* using antibodies that bound to a key defence receptor on the cells. They then exposed the cells to a pulse of an amino acid called glutamine that was labelled with an isotope, and traced its metabolism. As expected, the glutamine was converted into glutamate, a molecule that was then made into GABA by the enzyme glutamic acid decarboxylase (two versions of this enzyme are dubbed GAD65 and GAD67). Levels of labelled GABA in B cells, but not in T cells, increased following activation of the cell, and B cells secreted labelled GABA. The authors report that T cells do not express GAD65 or GAD67, whereas B cells express GAD67. Together, these results demonstrate that immune stimulation induces mouse and human B cells to synthesize and secrete GABA (Fig. 1).

Previous studies investigating autoimmunity found that T cells express type A GABA ($GABA_A$) receptors. Activation of these receptors through GABA binding opens a chloride channel in the receptor. This opening leads to the inhibition of inflammatory types of T cell called helper CD4 T cells and killer CD8 T cells[3–6], and both T-cell types are key contributors to tissue damage in autoimmune disease. Activation of $GABA_A$ receptors also boosts the numbers of a type of T cell called a regulatory T cell, which dampens inflammation[5,7]. Other cells of the immune system, called antigen-presenting cells, also have $GABA_A$ receptors. They include macrophages, dendritic cells and NK cells, which aid defence by presenting fragments of foreign proteins called antigens to T cells. The activation of $GABA_A$ receptors on these antigen-presenting cells reduces their pro-inflammatory properties[4,8,9]. Several laboratories have harnessed the anti-inflammatory actions of molecules that activate $GABA_A$ receptors to inhibit autoimmunity in mouse models of diseases such as type 1 diabetes, multiple sclerosis and rheumatoid arthritis, as well as to tackle severe disease

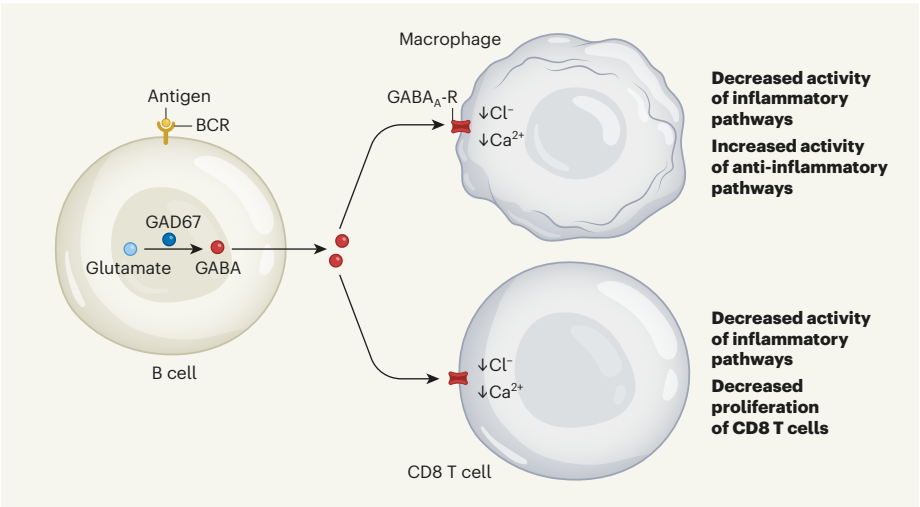

Figure 1 | Release of GABA by B cells inhibits antitumour responses of immune cells. Zhang *et al.*[1] report that mammalian immune cells called B cells secrete the molecule GABA, which is also a neurotransmitter in the brain. B cells synthesize GABA when they detect a protein fragment called an antigen (from a foreign protein, for example) through their B-cell receptor (BCR). These activated B cells use the enzyme GAD67 to convert the molecule glutamate into GABA, which is then secreted, presumably not within a vesicle, as occurs in neurons[13]. GABA can bind to and activate type A GABA receptors ($GABA_A$-Rs) on nearby immune cells, such as macrophages and CD8 T cells. This opens ion channels in the receptors, reducing the intracellular levels of calcium (Ca^{2+}) and chloride (Cl^-) ions. These changes dampen inflammatory pathways in, and the proliferation of, immune cells and boost anti-inflammatory pathways, thereby hindering antitumour immune responses.

caused by a coronavirus that infects mice[4–7,9].

GABA inhibits neurotransmission by preventing the neuronal depolarization process that is needed for signal transmission by neurons. By contrast, the activation of T-cell $GABA_A$ receptors leads to depolarization, which limits calcium entry into the cell and reduces cellular replication and inflammatory activities[10]. Extending those previous studies, Zhang and colleagues report evidence from *in vitro* experiments that a drug called muscimol that specifically activates $GABA_A$ receptors limits the activation and proliferation of CD8 T cells, whereas a $GABA_A$-receptor inhibitor called picrotoxin boosts calcium levels in mouse and human CD8 T cells.

The authors investigated whether GABA secreted by B cells affected antitumour responses *in vivo*. They focused on a type of cancer called colon carcinoma, and studied a mouse model of this tumour in which cancer growth is reduced in animals deficient in B cells, a finding that suggests that B-cell-derived factors limit antitumour responses in this model[11,12]. The authors report that tumours in B-cell-deficient mice had more tumour-infiltrating CD8 T cells than did tumours in control animals (tumour-bearing wild-type mice that had B cells). Moreover, the CD8 T cells in the B-cell-deficient mice had higher levels of molecules associated with cell-killing capacity (cytotoxic molecules) and with inflammation than did such T cells in the control mice.

GABA administration had no effect on tumour growth in wild-type mice, whereas in B-cell-deficient mice it increased the tumour to a size closer to that of tumours found in the wild-type mice. This GABA treatment lowered the number of tumour-infiltrating CD8 T cells and reduced the production of cytotoxic and inflammatory molecules in B-cell-deficient mice. Moreover, in control animals given a $GABA_A$-receptor-specific inhibitor, tumour size was reduced and the cytotoxicity of tumour-infiltrating CD8 T cells increased. These findings indicate that B-cell-secreted GABA dampens the response of tumour-cell-killing CD8 T cells *in vivo*.

Immune responses to tumours can sometimes fail to curb cancer growth because the inflammatory anticancer immune response in the tumour is dampened. Tumour-associated macrophages often have a role in this suppression. Continuing to work with the colon cancer mouse model, the authors observed that tumour-associated macrophages in B-cell-deficient mice showed enhanced expression of pro-inflammatory pathways compared with such macrophages in control mice. These pathways were reduced if the B-cell-deficient mice received GABA. Furthermore, tumour-associated macrophages isolated from wild-type mice that had received a $GABA_A$-receptor inhibitor showed increased expression of genes related to calcium signalling and inflammatory cytokines. GABA also enhanced the differentiation and numbers of macrophages that had anti-inflammatory characteristics. Hence, B-cell-secreted GABA can shift the antitumour responses of both tumour-associated macrophages and CD8 T cells towards less inflammatory activity.

Finally, the authors generated mice whose B cells did not express GAD67 and that were therefore GABA-deficient. Crucially, tumour cells implanted into these mice showed reduced growth, and the animals' tumour-infiltrating CD8 T cells had greater cytotoxicity and stronger pro-inflammatory properties than did tumour cells in control animals whose B cells expressed GAD67.

Together, Zhang and colleagues' evidence convincingly demonstrates the surprising finding that B cells secrete GABA, which can promote anti-inflammatory macrophages and inhibit the antitumour responses of CD8 T cells through their $GABA_A$ receptors. The results should stimulate further preclinical (animal) and clinical studies.

One question to be considered is whether GABA, which is widely consumed in various health supplements, could have adverse effects in humans. The authors found that GABA administration increased tumour size in B-cell-deficient mice to be more like that in wild-type mice, but did not affect tumour size in the wild-type mice. Currently, there have not been associations reported between GABA consumption in supplements, or the use of drugs that specifically enhance $GABA_A$-receptor activity — such as alprazolam (Xanax), which is used to treat anxiety disorders — and changes in tumour mass. However, this possibility should be examined. It will be of interest to elucidate the types of tumour for which B-cell-secreted GABA modulates antitumour responses, and whether this modulation occurs in the bone marrow, lymph nodes and tumour microenvironment.

Another area for future study will be to investigate the effect of B-cell-secreted GABA on pro-inflammatory CD4 T cells, and whether the response of regulatory T cells is enhanced in tumours[11] and in tissues that have become inflamed through autoimmune disease. Moreover, it would be worth further investigating pharmacological inhibition of immune-cell $GABA_A$ receptors, or the use of $GABA_A$-receptor-deficient T cells or antigen-presenting cells that have been modified to boost their attack on tumour cells (in what are known as adoptive-cell anticancer treatments), for their potential as therapeutic approaches. If promising evidence emerges from preclinical studies, such investigations could set the stage for clinical trials.

Daniel L. Kaufman is in the Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California 90095, USA.
e-mail: dkaufman@mednet.ucla.edu

1. Zhang, B. *et al. Nature* https://doi.org/10.1038/s41586-021-04082-1 (2021).
2. Deidda, G., Bozarth, I. F. & Cancedda, L. *Front. Cell. Neurosci.* **8**, 119 (2014).
3. Mendu, S. K. *et al. Mol. Immunol.* **48**, 399–407 (2011).
4. Tian, J., Song, M. & Kaufman, D. L. *Sci. Rep.* **11**, 5402 (2021).
5. Tian, J. *et al. ImmunoHorizons* **3**, 498–510 (2019).
6. Tian, J., Middleton, B. & Kaufman, D. L. *Viruses* **13**, 966 (2021).
7. Soltani, N. *et al. Proc. Natl Acad. Sci. USA* **108**, 11692–11697 (2011).
8. Bhat, R. *et al. Proc. Natl Acad. Sci. USA* **107**, 2580–2585 (2010).
9. Tian, J., Yong, J., Dang, H. & Kaufman, D. L. *Autoimmunity* **44**, 465–470 (2011).
10. Alam, S., Laughton, D. L., Walding, A. & Wolstenholme, A. J. *Mol. Immunol.* **43**, 1432–1442 (2006).
11. Zhang, Y., Morgan, R., Podack, E. R. & Rosenblatt, J. *Immunol. Res.* **57**, 115–124 (2013).
12. Akrami, M. *et al. Proc. Natl Acad. Sci. USA* **117**, 23674–23683 (2020).
13. Soghomonian, J.-J. & Martin, D. L. *Trends Pharmacol. Sci.* **19**, 500–505 (1998).

The author declares competing interests. See go.nature.com/3bbtr19 for details.