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Toxicological Sciences, 2024, **202(1)**, 1–12
https://doi.org/10.1093/toxsci/kfae114
Advance Access Publication Date: September 9, 2024
Contemporary Review

Exploring the interplay between cannabinoids and thymic functions

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Abstract

Cannabinoids, derived from the *Cannabis sativa* plant, have garnered increasing attention for their potential therapeutic applications in various diseases. The pharmacologically active compounds in *Cannabis*, such as delta-9-tetrahydrocannabinol and cannabidiol, exhibit diverse immunomodulatory properties. Although studies have explored the effects of cannabinoids on immune function, their specific interactions with the thymus, a primary immune organ critical for T-cell development and maturation, remain an intriguing area of investigation. As the thymus plays a fundamental role in shaping the immune repertoire, understanding the interplay between cannabinoids and thymic function may shed light on potential benefits or concerns associated with *Cannabis*-based therapies. This article aims to provide an overview of the current scientific knowledge regarding the impact of medicinal *Cannabis* on the thymus and its implications for disease treatment and immune health.

Keywords: *Cannabis* research; cannabidiol; tetrahydrocannabinol; thymus; thymocyte

Cannabinoids, the bioactive compounds found in *Cannabis*, have demonstrated compelling immunomodulatory properties, sparking considerable interest in their potential therapeutic applications. Extensive preclinical studies have revealed the impact of cannabinoids on diverse immune cell functions. In parallel, clinical trials have been initiated to investigate the effects of cannabinoids on the immune system in various health conditions. The objective of these trials primarily revolves around the evaluation of conditions and diseases linked to nervous and circulatory system disorders, including chronic pain, epilepsy, anxiety, hemorrhagic aneurysm, diabetes, cystic fibrosis, and various types of cancer. Although some trials have reported positive outcomes, the complex interactions between cannabinoids and the immune system require further investigation to understand their full therapeutic potential.

Beyond these, studies have primarily focused on pollen allergy, rheumatoid arthritis, and responses to infections like human immunodeficiency virus (HIV), malaria, COVID-19, and influenza. Indeed, a greater focus on immune system dysfunctions is necessary. Furthermore, given the importance of the thymus in T-cell maturation and immune tolerance, preclinical and clinical research should explore the interaction between cannabinoids and thymic function. From the survey of scientific findings relating to the effect of *Cannabis* extract or its compounds on the thymus, it is possible to predict the impact of the medicinal use of cannabinoids and their physiological repercussions on the thymus.

Cannabis and its compounds: overview of actions

For thousands of years, the *Cannabis sativa* plant has been used for medicinal, recreational, and spiritual purposes throughout the world (Turner et al. 2017; Rock and Parker 2021). Popularly known as marijuana, *C. sativa* is a species that belongs to the Cannabaceae family, originating in central Asia (Li 1973; Schilling et al. 2020). Recent discoveries point to a growing number of studies demonstrating the therapeutic potential of more than 550 compounds of this plant, such as cannabinoids, terpenoids, and flavonoids, including delta-9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), which are summarized in Table 1 (Mechoulam 2005; Ahmed et al. 2015).

THC is the most abundant chemical compound in *Cannabis*, discovered in the 1970s (ElSohly et al. 2017; Turner et al. 2017; Chayasirisobhon 2020). As the main psychoactive component in *Cannabis*, THC is responsible for causing the classic psychotropic effects mediated by the dopaminergic system that drive users to seek *Cannabis*, such as being "stoned" and "wanting more drug" (Curran et al. 2002; Hart et al. 2002; Bloomfield et al. 2016). THC can induce euphoria, disturbances in episodic and working memory, change in perception, high levels of anxiety, deficit in verbal and cognitive fluency, as well as positive and negative symptoms of schizophrenia (i.e. paranoia, delusions, and conceptual disorganization) (D'Souza et al. 2004; Morrison et al. 2009; Martin-Santos et al. 2012; Morgan et al. 2018).

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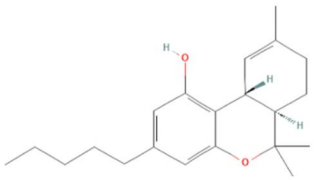
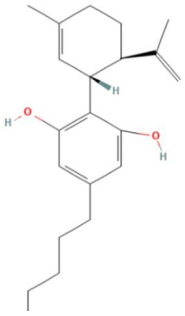
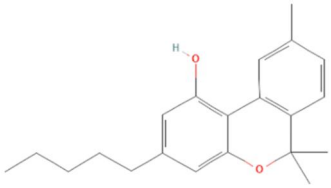
Table 1. Main *Cannabis* compounds, their chemical structures, and current use.

Cannabis compounds	Chemical structure	Current use	References
Delta-9-tetrahydrocannabinol (THC)		Classic psychotropic effects; Induces euphoria, disturbances in episodic and working memory, change in perception, high levels of anxiety, deficit in verbal and cognitive fluency, as well as positive and negative symptoms of schizophrenia.	(Curran et al. 2002; Hart et al. 2002; Bloomfield et al. 2016) (D'Souza et al. 2004; Morrison et al. 2009; Martin-Santos et al. 2012; Morgan et al. 2018)
Cannabidiol (CBD)		Treat seizures in Dravet syndrome, Lennox–Gastaut syndrome, and rare genetic forms of epilepsy; Treatment of spasticity in multiple sclerosis.	(Arzimanoglou et al. 2020; Golub and Reddy 2021; von Wrede et al. 2021) (Fraguas-Sánchez and Torres-Suárez 2018)
Cannabinol (CBN)		Inhibits oxytosis/ferroptosis in neurons; Prevents amyloid toxicity and aggregated Aβ accumulation; Antioxidant activity; Analgesic activity; Anti-inflammatory activity; Antibacterial activity; Orexigenic activity; Sleep induction.	(Liang et al. 2022) (Schubert et al. 2019) (Dawidowicz et al. 2021) (Wong and Cairns 2019) (Jan et al. 2003) (Appendino et al. 2008) (Farrimond et al. 2012a, 2012b) (Corroon 2021)

On the other hand, CBD is the main nonpsychoactive and nonintoxicating component in *Cannabis*, unable to lead to the "stoned" state observed following THC or *Cannabis* use (Curran et al. 2016; Englund et al. 2017). Previous studies have shown that CBD can promote opposite effects when compared with THC (Bhattacharyya et al. 2010; Batalla et al. 2014). CBD is the active component of the drug Epidiolex, used to treat seizures in Dravet syndrome, Lennox–Gastaut syndrome, and rare genetic forms of epilepsy (Fraguas-Sánchez and Torres-Suárez 2018; Arzimanoglou et al. 2020; Golub and Reddy 2021; von Wrede et al. 2021). Furthermore, this cannabinoid is found in a standardized extract (Sativex), combined with THC, and has been used in the treatment of spasticity in multiple sclerosis (Fraguas-Sánchez and Torres-Suárez 2018). Research has shown that CBD has tremendous therapeutic potential for many pathologies but can also have side effects (Huestis et al. 2019).

Similar to CBD, but in lower concentrations in *C. sativa* and extracts, CBN is a nonpsychoactive phytocannabinoid resulting from the degradation of THC (Pertwee 2006; Corroon 2021; Maioli et al. 2022). CBN inhibits oxytosis/ferroptosis in neurons by preserving mitochondrial functions and exerting a neuroprotective role (Liang et al. 2022). These authors demonstrated that these effects are mediated by a pathway independent of cannabinoid receptors (CBR). In a preclinical drug-screening study for Alzheimer's disease, CBN prevented amyloid toxicity, blocked cell death, and prevented aggregated Aβ accumulation, stimulating its degradation and removal from neurons (Schubert et al. 2019). Furthermore, CBN also exhibits antioxidant (Aiken et al. 2004; Dawidowicz et al. 2021), analgesic (Wong and Cairns 2019), anti-inflammatory (Elsohly et al. 1981; Jan et al. 2003), antibacterial (Appendino et al. 2008), and orexigenic (Farrimond et al. 2012a, 2012b) activities, as well as promotes sleep induction (Corroon 2021).

The primary CBR are type-1 (CB1R) and type-2 (CB2R) isoforms (Lutz 2020). These CBRs belong to the family of seven transmembrane G protein-coupled receptors. Because they essentially couple to inhibitory G proteins, these receptors inhibit adenylate cyclase and some voltage-sensitive calcium channels, as well as can stimulate mitogen-activated protein kinases (MAP kinases) and inwardly rectifying potassium channels (GIRKs) (Howlett et al. 2002). CB1R is widely expressed in several brain regions and other locations such as the intestine, liver, fat (adipocytes), pancreas, skeletal muscle, and immune cells (Cota et al. 2003; Osei-Hyiaman et al. 2005; Cavuoto et al. 2007; Cota 2007; Mackie 2008). CB2Rs have a more restricted distribution in immune cells, including T cells, B cells, macrophages, and microglia (Stella 2010; Cabral et al. 2015). Table 2 describes the main characteristics of both CBR.

Effects of *Cannabis* and cannabinoids on the immune system

The use of *Cannabis*, either by cigarettes or medicinal treatment, as well as its derivatives mentioned above, has shown several adverse effects, negatively impacting the immune system. However, the context of this immunosuppression can be beneficial in cases of inflammatory and autoimmune diseases, as well as in organ transplants. On the other hand, the weakening of the immune response is detrimental to defending against pathogens and tumors (Oláh et al. 2017). The effects of cannabinoids on the immune system can vary depending on the specific cannabinoid,

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its concentration, the exposure time, the type of immune cells involved, and the age and health status of individuals (Maggirwar and Khalsa 2021). *Cannabis* and specific cannabinoids' effects on immunological function have been extensively studied using in vitro models, often utilizing T cells, B cells, macrophages, and dendritic cells (DCs), which play crucial roles in immune responses (Fig. 1). However, in vitro studies have limitations because they do not fully capture the complexity of the human immune system and the intricacies of in vivo responses (Tabernilla et al. 2021).

Regarding innate immunity, there are reports on the effects of cannabinoids on natural killer (NK) cells because THC and CBD reduce the secretion of proinflammatory cytokines and decrease the cytolytic activities of human and mouse NK cells (Sarsembayeva et al. 2022). Under *Cannabis* smoke exposure, the number of neutrophils increases in the lungs (Haidar et al. 2023) and CBD induces apoptosis and elastase secretion in neutrophils (Bhat et al. 2023). Furthermore, it has already been described that cannabinoids substantially influence the function of macrophages and their polarization. For instance, Zaiachuk et al. (2023) demonstrated that THC and CBD attenuate cytokine release by macrophages in vitro. Also, CBD diminishes macrophage infiltration on the skin and liver in the fibrosis model (Del Río et al. 2022). Also, CBN downregulates proinflammatory gene transcription in macrophages (Gojani et al. 2023) and enhances IL-10 secretion by keratinocytes (Gu et al. 2019).

In the lymphoid compartment of acquired immunity, cannabinoids have demonstrated a deleterious effect on B cells, inducing apoptosis, altering the cytokine profile, and reducing the number of IgG- and IgM-secreting cells (Lampron et al. 2023). *Cannabis sativa* extracts inhibited the proliferation of T cells by CD25 suppression without causing apoptosis (Devi et al. 2022). Furthermore, CBD reduced $CD4^+$ T cells with proinflammatory profile and autoimmune $CD8^+$ T cells (González-Mariscal et al. 2022). On the other hand, cannabinoids can promote DCs with a tolerogenic profile and increase the frequency of regulatory T cells (Tregs), which has

Table 2. Cannabis receptors, localization, and their significance.

Cannabis receptors	Localization	Significance/use	References
Cannabinoid receptors type-1 (CB1R)	Brain Intestine Liver Fat (adipocytes) Pancreas Skeletal muscle Immune cells	Synaptic transmission Emotional processing and reward Motivation Motor activation cognitive function Neuroprotection Memory Synaptic plasticity	(Cota et al. 2003; Osei-Hyiaman et al. 2005; Cota 2007; Cavuoto et al. 2007; Mackie 2008; Viveros et al. 2012; Osborne et al. 2019; Martínez-García et al. 2021)
Cannabinoid receptors type-2 (CB2R)	Immune cells T cells B cells Macrophages Microglia	Anti-inflammatory role in neuropathologies	(Stella 2010; Cabral et al. 2015; Spiller et al. 2019; Tanaka et al. 2020)

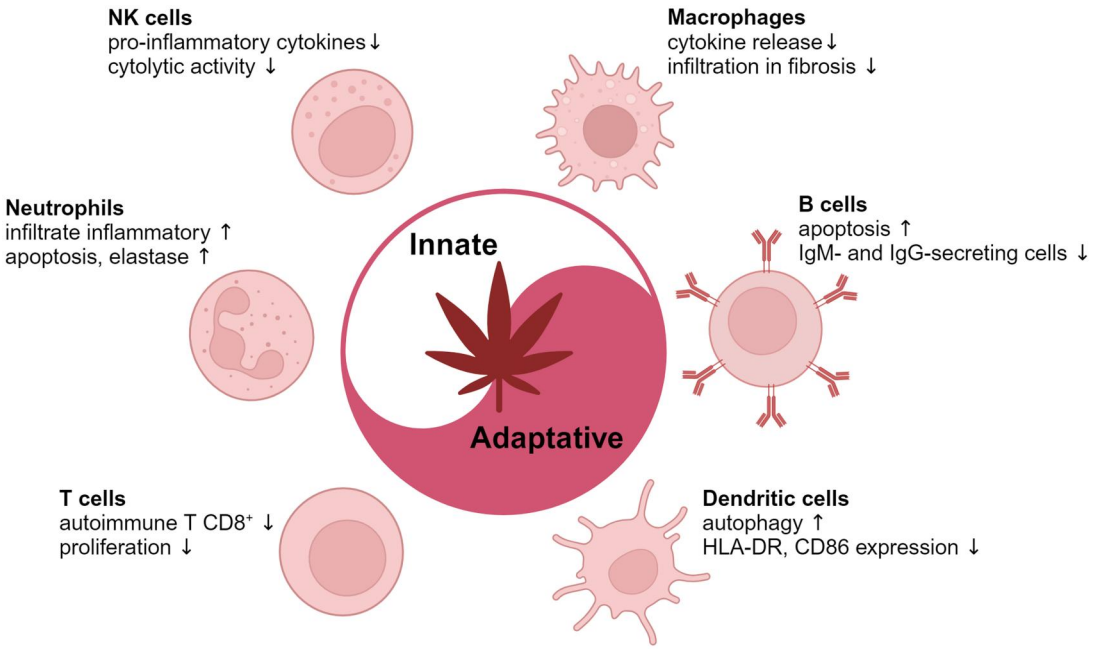

Fig. 1. Some actions of cannabinoids on immune cells. Here are the references used for the descriptions in the figure. The cells classified within innate immunity include NK cells (Sarsembayeva et al. 2022), neutrophils (Bhat et al. 2023; Haidar et al. 2023), and macrophages (del Río et al. 2022; Zaiachuk et al. 2023). Within adaptative immunity, the cells include DCs (Angelina et al. 2021), T cells (Devi et al. 2022; González-Mariscal et al. 2022), and B cells (Lampron et al. 2023).

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broad therapeutic appeal (Angelina et al. 2021). For that, it is important to discuss that studies investigating the direct impact of *Cannabis* use on human immune function, as measured by the number of T and B cell lymphocytes, macrophages, or immunoglobulin levels, have yielded mixed and sometimes conflicting results.

Thymus: structure and function

The thymus is a specialized organ of the immune system located in the superior and anterior mediastinum in front of the heart. It consists of two lobes, each shaped like an elongated pyramid, possessing a thin capsule of connective tissue. Blood vessels and nerves enter the organ through this capsule. The thymus is relatively large in infancy and early puberty; however, it regresses to a vestigial structure in old age. Despite this, it remains functional throughout life. The main thymic function is the development and maturation of T lymphocytes, which are crucial for immune responses (Tripathy et al. 2019; Hale et al. 2020).

Internally, the thymus is subdivided into two distinct histologic regions: the outer cortex and the inner medulla. The thymic cortex contains numerous lymphocytes, known as thymocytes, which are undergoing maturation. Also, the cortex contains thymic epithelial cells (TECs) that play a crucial role in T-cell development. The medulla, in turn, exhibits fewer thymocytes compared with the cortex; nevertheless, these are mature T cells that have completed their development. Medullary TECs and DCs contribute to eliminating self-reactive T cells through negative selection, promoting the establishment of immune tolerance. Lastly, the thymic extracellular matrix is abundant in collagen, fibronectin, and laminin, which support all the cells that populate this organ (Campinoti et al. 2020; Li et al. 2023).

Intrathymic thymocyte differentiation refers to the process by which precursor cells can become mature and functional T cells that can recognize foreign antigens while maintaining self-tolerance. The process begins with entering precursor cells into the thymus from the bone marrow. These thymocytes migrate from the outer cortex toward the inner medulla, interacting with various stromal cells (TECs and DCs, mainly) and acquiring distinct surface markers. Initially, thymocytes are known as double-negative (DN) cells because they lack the expression of both CD4 and CD8 co-receptors. DN thymocytes further differentiate, expressing a functional T-cell receptor (TCR), CD4, and CD8 markers in their membrane, now as double-positive (DP) thymocytes. These cells undergo further maturation to eliminate self-reactive T cells, and they differentiate into either $CD4^+$ or $CD8^+$ single-positive (SP) thymocytes. These mature T cells (SP thymocytes) exit the thymus and populate peripheral lymphoid organs, playing essential roles in immune responses (Ross et al. 2018; Singh et al. 2020).

Despite the importance of the thymus for the body's immunity, this organ is extremely sensitive to insults that can cause its atrophy, which refers to a reduction in its size and functional capacity. Thymic atrophy occurs due to various factors, including age-related involution (Yang et al. 2024), stress through glucocorticoids (Moleriu et al. 2014), infections (viruses like HIV and cytomegalovirus) (Guaraldi et al. 2019; Kielsen et al. 2024), some medical conditions (malnutrition and genetic disorders) (Nabukeera-Barungi et al. 2021; Gaiser et al. 2024), and exposure to certain drugs (Rengifo et al. 2024), radiation (Wang et al. 2024), or toxins (environmental pollutants) (Zhu et al. 2022) At the molecular level, thymic atrophy is attributed mainly to intense cellular depletion due to increased thymocyte apoptosis (activation of caspases) and interference in the thymocyte development (Kinsella and Dudakov 2020).

Cannabinoids and their effects on thymus

As several other natural products have already demonstrated beneficial or deleterious effects on the thymus, *Cannabis* extracts, THC, or CBD have already been evaluated in this regard by some studies. A search in the literature points to the first report (to the best of our knowledge) of the study published in 1974 by Roger G. Pertwee, a brilliant researcher who contributed to the present day with relevant research on cannabinoids. In his study, it was observed that the treatment with *Cannabis* extract, subcutaneously, reduced the relative weight of the mice thymus by 50% (Pertwee 1974). Additionally, the author considered different experimental conditions, separating the animals into groups exposed to social isolation or thermal variation (20°C or 30°C). It is worth noting that more studies have been performed since then to expand our understanding of the interaction between *Cannabis* compounds and thymus, exploring additional aspects such as immune response, cell proliferation, and molecular mechanisms.

A comprehensive search yielded a total of 20 articles. We have categorized these articles into five distinct topics, as demonstrated in Figure 2. These categories provide a systematic overview of the research conducted in this field. The main variables that have been analyzed concerning thymic function, based on their citation frequency, can be summarized as follows: (i) thymus weight or cellularity; (ii) intracellular signaling of thymocytes; (iii) proliferation or apoptosis; (iv) gene expression in the thymus; (v) others (additional aspects of thymic function that are not directly related to the previous variables). This categorization allows for a clearer understanding of the various aspects investigated, highlighting the diverse perspectives from which this topic has been approached.

Thymic atrophy refers to the shrinkage or decrease in size and functional capacity of the thymus. It can occur due to various factors, including aging, certain diseases, medications, and external influences. This reduction in thymic weight and cellularity can lead to (indirectly) a decline in T-cell production and function, resulting in a compromised immune system. This can make individuals more susceptible to infections, autoimmune disorders, and impaired immune responses against cancer cells. In some cases, thymic atrophy may be transient and reversible. After the cessation of certain medications or the removal of the underlying cause, the thymus can regain its size and functionality producing new T cells (Majumdar and Nandi 2018).

Thymus weight analysis, reflecting thymocyte cellularity, has consistently been a significant criterion for assessing the direct effects of cannabinoids on the thymus. However, it is important to note that experimental models, routes of administration, timing, and dosage have varied considerably across studies. Despite these variations, the majority of results indicate that cannabinoids induce thymic atrophy (Pertwee 1974; Baczynsky and Zimmerman 1983; Benevenuto et al. 2017; Chen et al. 2017) and a reduction in all thymocyte subsets (McKallip et al. 2002; Lombard et al. 2011). Nevertheless, there have been studies that reported no alterations in the size and cellularity of the thymus following cannabinoid exposure (Bhargava et al. 1996; del Arco et al. 2000). Additionally, a single study revealed an increase in thymus weight and the number of thymocytes, potentially associated with reduced testosterone levels in the animals (Murphy et al. 1995).

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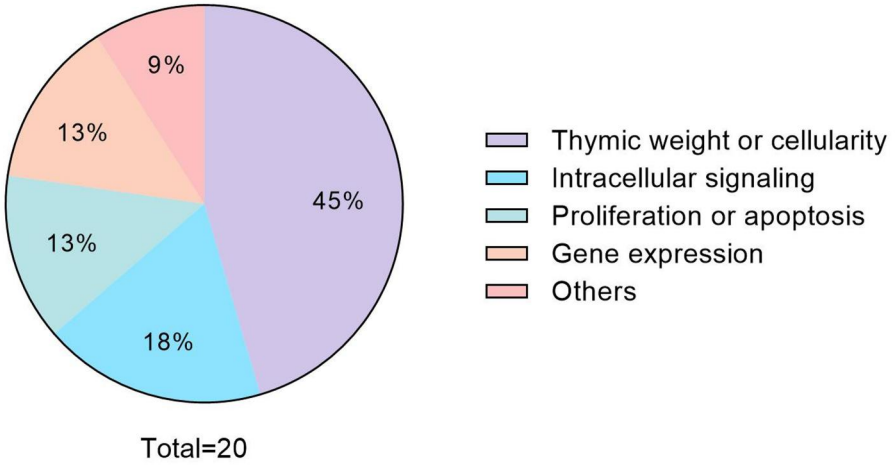

Fig. 2. Cannabis and thymus studies. The number of studies about Cannabis and the thymus totals 20. They were categorized into topics such as thymic weight and cellularity, intracellular signaling, proliferation or apoptosis, gene expression, and other subjects. The percentages in each topic describe the frequency with which these themes are addressed in each study.

The second category, focusing on intracellular signaling, aligns with the findings related to proliferation and gene expression in thymocytes, which will be discussed in subsequent categories. This category includes in vitro experiments utilizing fresh thymocytes treated with cannabinoids. A study has shown that THC can inhibit calcium mobilization in Concanavalin A (ConA)-induced mouse thymocytes, affecting calcium influx and intracellular release (Yebra et al. 1992). After that, a research group has demonstrated that CBN can impede adenylate cyclase, as well as the DNA binding activity of protein kinase A (PKA) and cAMP response element-binding protein/activating transcription factor (CREB/ATF) (Herring et al. 1998). Furthermore, CBN inhibited various signaling pathways, including factor nuclear kappa B (NF-κB), extracellular signal-regulated kinases (ERK), and MAP kinases in activated thymocytes (Herring et al. 2001). By exerting these effects, cannabinoids have the potential to influence critical signaling pathways that regulate thymocyte development and maturation and, subsequently, effector T-cell functions in the periphery of the immune system.

The subsequent categories, namely proliferation/apoptosis and gene expression, encompass studies that contribute to our understanding of the primary effect of cannabinoids, which is thymic atrophy. By analyzing changes in gene expression and in the processes of cell proliferation/death, researchers have identified specific genes that are up or downregulated following cannabinoid exposure and their relationship with thymocyte apoptosis, ultimately impacting thymic function and homeostasis. Maintaining thymic homeostasis is essential for ensuring the production of a diverse and functional T-cell repertoire capable of recognizing and responding to a wide range of pathogens and the immune surveillance against cancer cells (Thapa and Farber 2019).

In this sense, Pross et al. (1987) observed that THC (or its metabolic product, 11-OH-THC) suppressed the proliferation of murine thymocytes stimulated with phytohemagglutinin (PHA) or ConA. A subsequent study demonstrated similar antiproliferative effects with CBN treatment, including a reduction in the secretion of interleukin-2 (IL-2, a mitogenic cytokine) by phorbol myristate acetate-activated thymocytes (Herring and Kaminski 1999). To endorse this fact, CBD-induced apoptosis was evidenced by cell cycle analyses in thymocytes (Lee et al. 2008). These observations were further supported by the identification of CB2Rs in thymic transcripts by Schatz et al. (1997), since in mice injected with a caspase inhibitor (McKallip et al. 2002) or with CB1/CB2 antagonists (Lombard et al. 2011), the effect of THC on thymic atrophy and thymocyte apoptosis was partially reversed. Interestingly, Xiong et al. (2022) investigated the impact of CB2R ablation specifically in the thymus and found an increase in intrathymic positive selection, which increased the numbers of mature $CD4^+$ and $CD8^+$ thymocytes. Also, Esain et al. (2015) showed that CB2R-signalling promotes thymic colonization by cell progenitors dependent on P-selectin expression. Collectively, these studies demonstrate that cannabinoids, including THC, CBN, and CBD, as well as their receptors, be, and CBD, can modulate thymic function and homeostasis.

In the last category of studies, specific hypotheses did not directly align with the previously discussed topics. Bailey et al. (1987) conducted a study assessing THC levels in fetuses of *Rhesus* monkeys. They observed higher levels of THC in fetal thymic homogenate compared with maternal and fetal plasma. This finding suggests the potential transfer of THC to the developing thymus during pregnancy and highlights the need for further investigations. Another study within this category focused on the effects of CBD on reactive oxygen species (ROS) production by thymocytes. Lee et al. (2008) demonstrated that treatment with CBD led to an increase in ROS generation by thymocytes, which can trigger apoptosis in these cells. Figure 3 and Table 3 summarize the findings of these articles.

In addition to the aforementioned studies, discussing two articles presenting conflicting findings is important. Jordà et al. (2002) conducted an in vitro study to evaluate the influence of natural and synthetic endocannabinoids on thymocyte migration. However, they reported that no significant migration was evident. Perhaps the quantification method of migrating thymocytes was not sensitive enough to detect them. Another study by Linher-Melville et al. (2020) employed an animal model of nerve injury and administered oral CBD and THC treatment for 14 d. Interestingly, they found an increase in thymic RNA expression of IL-2, IL-4, IL-17, interferon-γ (IFN-γ), and tumor necrosis factor-α, but only in male animals. These findings suggest a potential gender-specific effect of CBD and THC on thymic immune response and an increase in cytokine production. Considering

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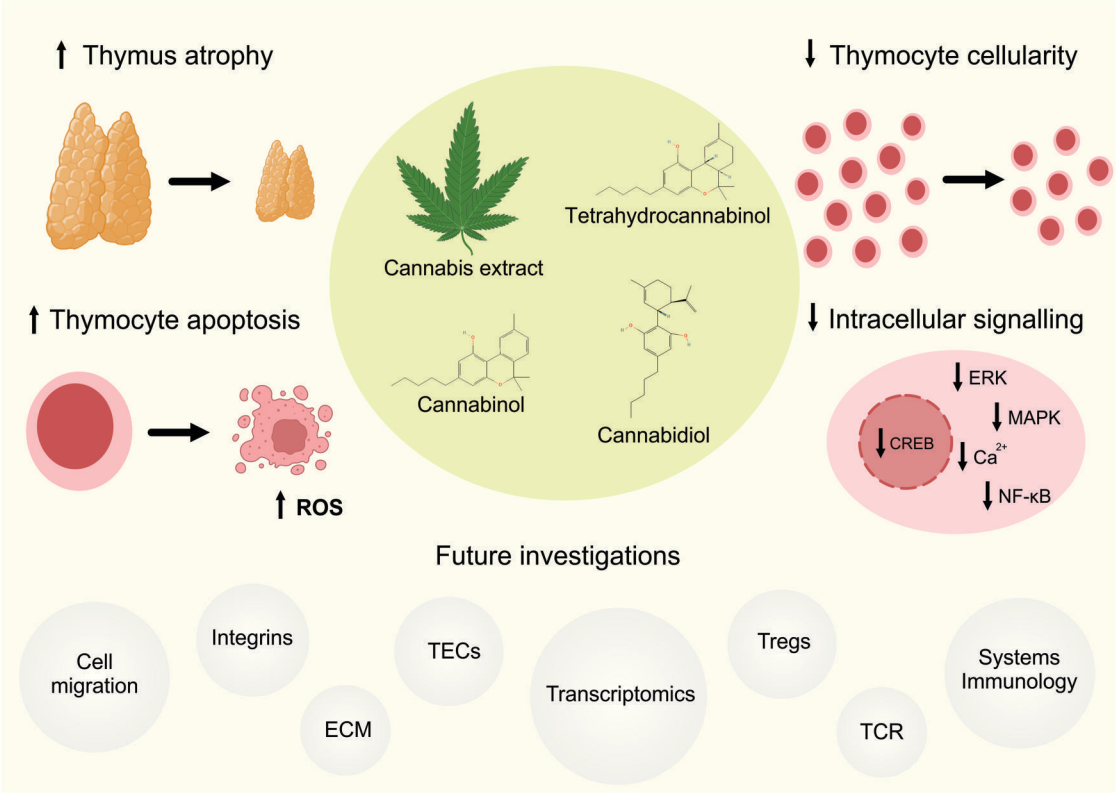

Fig. 3. The cartoon depicts the main effects of *Cannabis* extract and its cannabinoids on thymic functions. *Cannabis* extract, delta-9-tetrahydrocannabinol, cannabidiol, and cannabinol can promote thymic atrophy and thymocyte apoptosis, reduce thymocyte cellularity, and downregulate some signaling pathways. Further investigations need to be carried out on these topics: cell migration, integrin, and extracellular matrix (ECM), thymic epithelial cells (TECs) and transcriptomics, regulatory T cells (Tregs) and T-cell receptor (TCR), and systems immunology as forefront in these research areas.

factors such as experimental conditions to interpret these results is important.

Future directions on thymus and *Cannabis* research

Since the first report, 50 years have passed on this research topic. In our view, several areas were well covered and established. However, there are still specific questions to be answered. To deepen the knowledge about the effects of *Cannabis* and its derivatives on the thymus, some suggestions for areas and experiments for future researchers are discussed. In addition to the classic analysis of the relative weight of the thymus and its cellularity, as well as phenotyping thymocyte subsets, there was no evaluation at the tissue level, with conventional histology to observe changes in the microarchitecture of the thymus, its cortical and medullary regions, and the corticomedullary junction. Also, immunohistochemical techniques can be used to evaluate the elements of the thymic extracellular matrix, verifying the presence and abundance of collagen, fibronectin, and laminin, as well as its major ligands, integrins (Mikušová et al. 2017).

At the cell level, the thymocyte could be better evaluated for its functionalities in vitro. Cytokine production assays, heterocellular or matrix adhesion, and vertical migration assays are great tools for these studies. It is necessary to highlight the analysis of other thymic cells present in the stroma of the organ, mainly TECs. Comprehensive analyses of its morphology, secretion of soluble and matrix proteins, and analysis of its transcripts (RNA sequencing) are highly recommended. These cells in a 3D culture system, as in Fetal Thymic Organ Cultures (FTOC) - done by Lombard et al. (2011), and the construction of thymic organoids or decellularized thymus scaffolds represent cutting-edge approaches in modern medicine. Furthermore, it would be possible to detail the generation of conventional T cells and Tregs and analyze the TCR repertoire through humanized or genetically modified animal models. Schmöle et al. (2015) developed a transgenic mouse for CB2-GFP, demonstrating its moderate expression in the thymus, allowing the physiological and pathological understanding of this receptor in the immune system. These valuable platforms provide a more clinically relevant context and will contribute to the broader field of cannabinoid research (Bortolomai et al. 2019; Campinoti et al. 2020; Haunerdinger et al. 2021).

Although murine studies have provided a wealth of knowledge to assess thymic function, clinical studies pose a challenge to truly measure thymus physiology. In humans, imaging techniques can be employed to estimate thymic function based on thymic size, such as X-ray or more advanced imaging techniques, such as computed tomography and magnetic resonance imaging. These noninvasive imaging methods allow for the assessment of thymic volume or thymic index, a ratio of thymic size to body surface area or body weight (Kerpel et al. 2019). These clinical assessments provide indirect measures of thymic function and have limitations because they do not provide a comprehensive understanding of the complex processes occurring within the thymus. However, they serve as a useful parameter that can be correlated with thymic function.

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Table 3. Main outcomes of *Cannabis*-treatment on thymus.

Substance	Dose/duration	Route	Model	Major effects	Reference
***Cannabis* extract**	100 to 500 mg/kg, 3 to 5 d	Subcutaneous	Tuck number 1 strain mice	Reduction on 50% of thymus weight	(Pertwee 1974)
	Cannabis smoke, 12 d	Airways	Balb/c mice	Reduction on 40% of thymus weight	(Benevenuto et al. 2017)
	200 to 400 mg/kg, 14 wk	Intragastrical	Sprague–Dawley rats	Reduction on 40% of thymus weight	(Chen et al. 2017)
THC	25 mg/ml, 14 d	Oral	Sprague–Dawley rats	Increase of proinflammatory genes in thymus (IL-2, IL-4, IL-17, IFN-γ)	(Linher-Melville et al. 2020)
	5 to 15 mg/kg, 4 d	Intraperitoneal	CD1 mice	Impairment of thymic weight and cellularity in 40%	(Baczynsky and Zimmerman 1983)
	3 to 10 μg/ml, 48 hr	In vitro	Balb/c mice	Suppression of thymocyte proliferation in 95%	(Pross et al. 1987)
	0,3 mg/kg, single dose	Intravenous	*Rhesus* monkey	Higher levels of THC in fetal thymus at 200 ng/g	(Bailey et al. 1987)
	4 to 20 μg/ml, 10 min	In vitro	Balb/c mice	Inhibition (35%) of Ca^{+2} mobilization on thymocytes	(Yebra et al. 1992)
	5 mg/kg, 1 wk	Oral	Sprague–Dawley rats	Increase (15%) in thymic weight and cellularity	(Murphy et al. 1995)
	10 mg/kg, 4 d	Subcutaneous	B6C3F1 mice	Thymus weight and cellularity were unaffected	(Bhargava et al. 1996)
	1 to 20 μM, 24 hr, and 1 to 50 mg/kg, 4 to 72 hr	In vitro, intraperitoneal	C57BL/6 mice	Increase (3×) in thymocyte apoptosis, reduction (70%) in thymic weight, and cellularity of all thymocyte subsets	(McKallip et al. 2002)
	20 to 50 mg/kg, 6 to 21 d	Intraperitoneal	C57BL/6 mice	Reduction (75%) in thymic cellularity and in all thymocyte subsets, increase (28%) of thymic apoptosis	(Lombard et al. 2011)
CBD	4 to 16 μM, 1 to 24 hr	In vitro	Balb/c mice	Increase of apoptosis (4×) and ROS production (2×) on thymocytes	(Lee et al. 2008)
CBN	1 to 20 μM, 10 min	In vitro	B6C3F1 mice	Inhibition of adenylate cyclase (80%), PKA, and CREB/ATF DNA binding activity	(Herring et al. 1998)
	1 to 25 μM, 10 min	In vitro	B6C3F1 mice	Reduction (50%) of IL-2 secretion by thymocytes and NF-kB Activation	(Herring and Kaminski 1999)
	5 to 15 μM, 15 min	In vitro	B6C3F1 mice	Inhibition of CREB, NF-kB, and ERK MAP kinases	(Herring et al. 2001)

Additionally, flow cytometry can be employed to analyze the distribution of T-cell subsets, including naive and memory T cells, which can indirectly reflect thymic function. Finally, the measuring of the output of newly produced T cells, known as recent thymic emigrants, is done through the quantification of T-cell receptor excision circles (TRECs), which are small circular DNA molecules formed during T-cell development inside the thymus. Quantifying TRECs in peripheral blood lymphocytes estimates thymic output (Sommer et al. 2020). Therefore, combining imaging techniques with other functional assessments, such as TREC analysis or flow cytometry, can provide a more comprehensive evaluation of thymic function. This multidimensional approach allows for a better understanding of the relationship between thymic size and its functional capacity during exposure to cannabinoids.

Recently, evidence suggested that the thymus remains functional and plays a crucial role in adults (Kooshesh et al. 2023). Studies indicate that an active thymus is associated with a higher survival rate and a reduced risk of developing cancer. Furthermore, it has been observed that thymectomy, which is the surgical removal of the thymus, results in a significant decrease in the number of $CD4^+$ and $CD8^+$ T cells in the peripheral blood of patients, which are crucial for the adaptive immune response. Reducing the number of these cells can compromise the immune system's ability to combat infections and diseases. Therefore, not only is it important to monitor thymic function in terms of naive T-cell export, but there is also a need for more clinical studies focusing on *Cannabis* users (both recreational and medicinal) and the incidence or occurrence of increased infections or other chronic health conditions due to T-cell deficiencies, especially in response to vaccines.

Finally, adults with an involuted thymus exhibited elevated levels of proinflammatory cytokines in the plasma, indicating an immune imbalance that may contribute to a higher susceptibility to inflammatory and autoimmune diseases. In this context, fungal infections are exacerbated in *Cannabis* users (Mohammed et al. 2024), which should be further explored when evaluating other viral and bacterial infections or malignancies. Future

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research could determine whether gene or cell therapies could restore thymus function in individuals who have had it removed.

Conclusion

Cannabis and its cannabinoids have been shown to exert immunosuppressive effects on the thymus, interfering with thymocyte development, thymic cellularity, and impaired immune function. Additionally, cannabinoids have been linked to increased thymocyte apoptosis, further contributing to thymic atrophy and disruption of the T-cell repertoire. Although the immunosuppressive properties of *Cannabis* hold potential in specific clinical settings, such as autoimmune diseases or organ transplant recipients, caution is warranted in the context of *Cannabis*-based therapies. Prolonged or excessive *Cannabis* use may compromise overall immune competence, increasing susceptibility to infections and impairing immune responses against tumors. Furthermore, the intricate balance between immune regulation and defense mechanisms demands careful evaluation of the risks and benefits associated with cannabis-based treatments, necessitating further research and evidence-based medical guidance to ensure safe and effective therapeutic use.

Acknowledgments

The authors would like to thank their mentors and eternal advisors for all their commitment to improving them as scientists and guiding them to follow in their footsteps in the academic career, researching thymus and cannabinoids. The authors would also like to extend their sincere gratitude to Salete Smaniotto and Olagide Wagner Castro.

Author contributions

Marvin Paulo Lins and Igor Santana de Melo have equal participation in the conception of the idea, writing, and discussion of this article.

Funding

Not applicable.

Conflicts of interest. The authors have no conflict of interest.

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Toxicological Sciences, 2024, 202, 1–12
https://doi.org/10.1093/toxsci/kfae114
Contemporary Review