MINIREVIEW

Δ^9-Tetrahydrocannabinol, Cytokines, and Immunity to *Legionella pneumophila* (43897A)

THOMAS W. KLEIN, CATHY NEWTON, WEIGANG ZHU, YEHIA DAAKA, AND HERMAN FRIEDMAN[1]

Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa, Florida 33612

Abstract. **The major psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (THC), has been shown to suppress the functions of various immune cells. However, the relationship of these findings to THC-induced suppression of host resistance to infection has not been firmly established. In this report, we review the literature concerning THC's effects on cytokine production and resistance to infection with *Legionella pneumophila* (Lp). Recent reports have linked THC-induced immunomodulation with drug-induced modulation of the cytokine network. Specifically, THC *in vivo* suppresses interferon (IFN) production while *in vitro* modulates the production of tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-2 (IL-2), and interleukin-2 receptor (IL-2R). These results suggested that THC treatment might alter host immunity by disrupting the cytokine network. Immunity and resistance to infection with Lp depends upon the activation of killer cells and the stimulation of the cytokine network. THC injection into rodents was observed to augment acute phase cytokine mobilization in response to a primary Lp infection; on the other hand, the drug suppressed the development of protective immunity and resistance to secondary Lp infection by causing a change in the profile of T helper cell cytokines produced by Th1 and Th2 cells. Thus, it appears that THC injection suppresses resistance to Lp infection by disrupting the cytokine network. Regarding the molecular mechanisms of these effects of THC, data is reviewed concerning the role of cannabinoid receptors (CR) in cells of the immune system. In summary, the literature to date supports the role of THC as an immunomodulator capable of suppressing resistance to infection through mechanisms involving alteration of the cytokine network. The role of CR receptors in these events has yet to be determined.** [P.S.E.B.M. 1995, Vol 209]

The immune system is a complex network of cells and cytokines with a primary function of preventing infection. However, as well controlled and resilient as the immune system is, many reports have documented the negative effect on immunity of environmental factors such as drugs of abuse. For example, opiates, cocaine, alcohol, and marijuana have all been reported to suppress the immune function. With regard to marijuana and immunity, most studies have been performed using Δ^9-tetrahydrocannabinol (THC). This polycyclic, aromatic hydrocarbon is one of over 60 cannabinoids present in marijuana smoke (1) and is the primary chemical ingredient with psychoactivity (Fig. 1). THC, when either injected into rodents or added to cultures of rodent or human immune cells, has been shown to suppress immune functions such as lymphocyte proliferation, antibody production, natural killer cell activity, and mac-

[1] To whom requests for reprints should be addressed at Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa, FL 33612.

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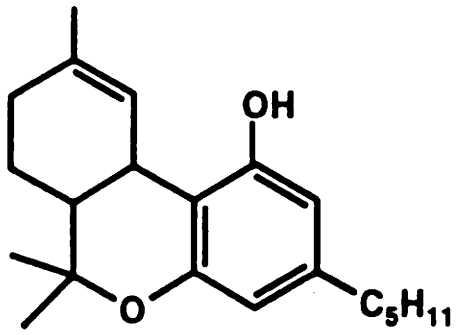

Figure 1. Chemical structure of Δ^9-tetrahydrocannabinol.

rophage activities (2–6). However, although these reports are compelling, they have had little impact on public health policy because, for the most part, they were obtained using drug concentrations considered outside the range of the human experience. In addition, the transition from *in vitro* culture systems to *in vivo* infection models has been slow. Thus, the purpose of this review is to describe recent information concerning the effects of THC on bacterial infection and relate this to effects of the drug on cytokine production and involvement of cannabinoid receptors.

The Effects of THC on Cytokine Production

Cytokines have assumed an ever increasing position of importance in the immune response since the discovery of these protein mediators three decades ago. Cytokines are known to be produced by immune cells, as well as many other cells in the body, including brain and endocrine cells. Cytokines mediate intercellular communication and therefore regulate immunity and inflammation during host defense against microbes and other non-self antigens. Several years ago, reports began to appear suggesting a link between THC-induced suppression of immunity and alterations in the cytokine network. It was found that THC treatment of mouse splenocyte cultures resulted in suppression of mitogen-induced interferon (IFN) production (7). Furthermore, splenocytes from drug-treated mice were found to produce much less IFN when stimulated in culture with mitogens. It was also reported that serum IFN levels were depressed following THC injection into rodents, indicating that this drug could suppress the formation of a cytokine important in resistance to viral infection (8, 9).

Another cytokine of major importance in immune regulation and host resistance is tumor necrosis factor (TNF). Like IFN, THC was reported to decrease the supernatant level of TNF-α in both primary murine, macrophage cultures (10) and murine, macrophage cell lines such as RAW264.7 (11). Additional studies showed THC treatment did not decrease the levels of TNF mRNA or the 26-kDa intracellular precursor of TNF but decreased instead the processing of precursor protein to the 17-kDa secreted form of the cytokine (11). THC treatment, however, does not always lead to a decrease in cytokine levels. For example, THC treatment was reported to increase rather than decrease supernatant interleukin-1 (IL-1) activity in cultures of mouse peritoneal macrophages co-treated with endotoxin (4) and the drug increased the processing and release of IL-1 rather than increasing cellular production (12). TNF-α was also shown to be increased rather than decreased by drug treatment when macrophage cell lines other than RAW264.7 were used (13). Although the above results are conflicting, they do suggest that THC treatment of various cell types can significantly alter the complex process of acute-phase cytokine production and processing.

A consistent observation in studies involving THC and lymphocyte cultures is that THC suppresses lymphoproliferation. The proliferation of T lymphocytes is regulated in part by signals generated through the IL-2/interleukin-2 receptor (IL-2R) system. Several years ago, it was found that THC suppressed IL-2-dependent lymphocyte proliferation as well as IL-2 activation of NK cells (14). This suggested that drug treatment compromised the IL-2/IL-2R system. For example, it seemed possible that THC could either reduce the autocrine production of IL-2 or suppress the number and signal transducing capabilities of IL-2R. Regarding the former possibility, it was found that THC modulated IL-2 production in splenocyte cultures (15). Regarding the latter possibility, it was found that THC suppressed the number of high and intermediate affinity IL-2 binding sites, as measured by equilibrium binding studies, in the NK-like cell line, NKB61A2 (16).

The IL-2R is composed of at least three different proteins, IL-2Rα, -β, and -γ. The above studies suggested THC treatment might be suppressing the production and/or cell surface expression of one or all of these proteins. However, preliminary flow cytometry experiments using antibodies to IL-2Rα proteins showed that treatment of NKB61A2 cells with THC increased rather than decreased the cell surface expression of these proteins (16). In support of this, recent Northern blotting studies using probes for IL-2Rα and -β mRNA show that the steady-state levels of the mRNAs are increased by THC treatment (Fig. 2). However, probing with the cDNA for IL2Rγ mRNA shows a decrease in the steady-state level with increasing THC concentration. Although further studies analyzing receptor protein content are needed, these preliminary results suggest THC treatment could lead to an alteration in the relative production and expression of receptor component proteins resulting in a suppression of the trimolecular receptor complex and

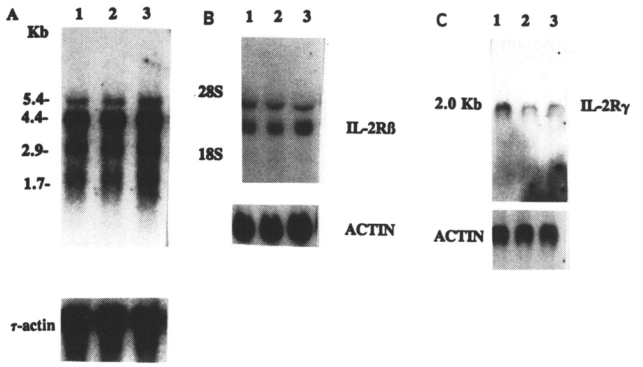

Figure 2. THC treatment increases IL-2 receptor α- and β-chain mRNA, but decreases IL-2 receptor γ-chain mRNA. NKB61A2 cells were incubated with IL-2 and either DMSO or THC for 4 hr. The cellular mRNA was extracted and probed with either the cDNA (kindly provided by Dr. Tasuku Honjo, Kyoto University) for the mouse IL-2Rα message (Panel A), the cDNA (kindly provided by Dr. Tadatsugu Taniguchi, Osaka University) for the mouse IL-2Rβ message (Panel B), or the cDNA (kindly provided by Dr. Kazuo Sugamura, Tohoku University) for the mouse IL-2Rγ message. All membranes were stripped and reprobed with an oligonucleotide probe for γ-actin. (A) Lane 1, DMSO (0.5%); Lane 2, THC (5 μg/ml); Lane 3, THC (10 μg/ml). (B) Lane 1, DMSO; Lane 2, THC (5 μg/ml); Lane 3, THC (10 μg/ml). (C) Lane 1, DMSO; Lane 2, THC (5 μg/ml); Lane 3, THC (10 μg/ml).

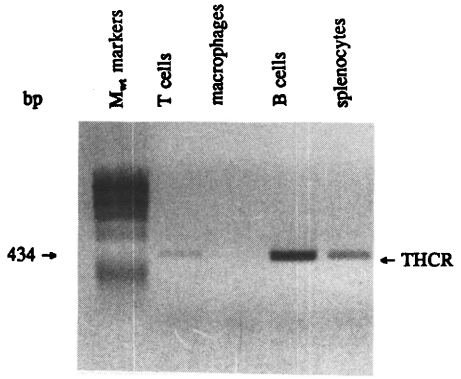

Figure 3. Immune cell subpopulations express different resting levels of THC receptor (THCR) mRNA. Mouse splenic T and B cells were purified by panning and peritoneal macrophages purified by plastic adherence. Total, cellular RNA was reversed transcribed and THCR cDNA fragments amplified by polymerase chain reaction using primers based on the sequence of the brain receptor cDNA. These primers amplify a product of 434 bp.

a decrease in the functional expression of the high-affinity IL-2R complex.

Cannabinoid Receptors and Immunity

Cannabinoids such as THC are believed to influence cell function through the action of specific cannabinoid receptors (CR) as well as by receptor independent mechanisms (17). CR were first definitively demonstrated in rat brain membrane preparations by showing stereospecific binding of various cannabinoid agonists (18). Agonist binding studies also showed inhibition of cAMP accumulation by means of a G_i protein (19). The cDNA encoding the binding activity was cloned in 1990 from a rat cerebral cortex cDNA library using a probe derived from the sequence of bovine substance-K receptor (20). Although the CR protein was not isolated and characterized, the translated sequence of the cDNA suggested a protein of 473 amino acids in length with several transmembrane regions, and to be a member of the G-protein-coupled family of receptors (20). The human counterpart of this gene was similarly cloned from a human brain cDNA library using a probe based upon conserved sequences in the G-protein receptor family (21). The deduced amino acid sequence suggested a protein of 472 residues that was 97% identical to the rat brain protein and 100% identical in the transmembrane regions. Expression of the gene was detected in brain and testes but not in other peripheral tissues. Recently, a second CR gene associated with the immune system has been cloned from a human leukemic cell line (HL60) cDNA library, using a probe based upon the G-protein receptor family (22). The deduced amino acid sequence was found to be only 44% identical to the brain receptor and, unlike the brain gene, was not expressed in the brain but rather in macrophages in the spleen. This and the fact that the order of binding potency of various receptor agonists differed from the brain receptor suggest the possibility that at least two CR exist and the distribution is tissue specific. Multiple receptors have also been suggested by the demonstration of a cannabinoid-regulated, N-type calcium channel, which is coupled to a pertussis toxin sensitive G-protein but is independent of intracellular cAMP (23). Also, a role for CR in arachidonic acid release involving phospholipases and G-proteins has been reported (24). This effect on arachidonic acid release may have some importance in drug effects on cytokine production, since eicosinoid metabolites are known to regulate cytokine production.

Cannabinoid effects on cells are also believed to occur by CR-independent mechanisms. The high lipid solubility of cannabinoids, which promotes the partitioning of these substances into cell membranes, may represent a means by which cannabinoids disrupt the function of membrane proteins and therefore cell function (25). These effects would be expected to occur at drug concentrations higher than those causing receptor-mediated changes and, indeed, this has been observed in CHO cells transfected with CR cDNA (26). Stereoselectivity was demonstrated for both cannabinoid binding and inhibition of cAMP accumulation, but not for the release of arachidonic acid and intracellular calcium. The IC_{50} doses for inhibition of cAMP accumulation were in the low nanomolar range, while the release of arachidonic acid and calcium required doses higher than micromolar and also occurred in untransfected CHO cells. Although these re-

sults are compelling, the interpretation may be an oversimplification in that the failure to demonstrate receptor coupling to functions such as arachidonic acid release may stem from the possibility that CHO cells are deficient in signaling pathways coupled to the cannabinoid receptor.

The recent demonstration of cannabinoid binding sites and CR gene expression in lymphoid tissue has raised important questions concerning the role of CR in immune cell function and immunomodulation by THC. Mouse splenocytes display specific binding sites for the synthetic cannabinoid, CP55,940, characterized by a single binding site, a K_d of 910 p*M*, and B_{max} of 1000 sites/cells (27). In addition, mRNA of the brain CR gene was demonstrated in splenocyte preparation using RT-PCR and structure-activity studies showed a correlation between suppression of the *in vitro* antibody response and the potency of various cannabimimetic agents (27). Human leukocytes have also been found to contain CR transcripts of the brain gene (28). Using RT-PCR with human CR primers, message was found in human spleen, tonsils, and blood leukocytes, with B cells showing the highest mRNA level and CD4 T cells the lowest. Several leukocyte cell lines were also shown to express the brain CR mRNA, including Daudi and THP1, but not Jurkat cells (28). B cells from mouse spleen also contain higher amounts of brain gene mRNA than do purified T cells or macrophages (Fig. 3). Furthermore, stimulated leukocytes express higher levels of CR mRNA than unstimulated ones. For example, Figure 4 shows results with the mouse macrophage cell line, RAW264.7. In these studies, the cells were stimulated with endotoxin and the cellular extracts monitored for 24 hr after stimulation. The results show the CR mRNA increased to a maximum at 6 hr and then declined by 24 hr, suggesting that the brain CR gene is turned on and off during leukocyte activation. Thus, it seems possible that the level of CR gene expression varies from one leukocyte subset to the next and further that gene expression varies with the extent of cell activation.

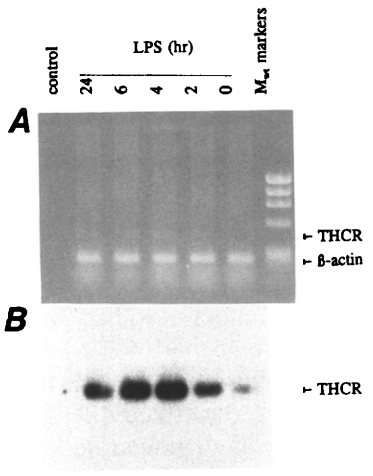

Figure 4. THCR mRNA increases with time following endotoxin stimulation. The mouse macrophage cell line, RAW 264.7, was cultured with endotoxin (1 μg/ml) for up to 24 hr, and the total RNA extracted at various times and subjected to RT-PCR using primers for the brain THCR cDNA (see legend Fig. 3). (A) The ethidium bromide gel of the various PCR products; (B) the Southern blot of the products using a cDNA probe for the brain receptor.

THC and Infection Models

It is clear from the above that drugs of abuse, such as THC, have the potential to disrupt the cytokine network when added to cultured immune cells. However, the relationship of these effects on cytokine production to drug effects on host resistance to infection is still unclear. In this regard, several reports appeared in the 1970s suggesting a possible link between marijuana consumption and increased susceptibility to infection. For example, it was reported that endotoxin could synergistically interact with THC when injected into mice, resulting in enhanced mortality (29). Similarly, it was reported that injection of cannabinoids, including THC, into mice made the animals more susceptible to infection with either *Listeria monocytogenes* or herpes simplex virus (30). Antimicrobial defense mechanisms of alveolar macrophages were also found compromised in various experimental models (31, 32). Several other studies reported the effect of THC on resistance to viral infection. For example, injection of THC into guinea pigs shortly before and after vaginal infection with herpes simplex virus resulted in greater morbidity and mortality (9). Furthermore, THC injection into mice decreased the resistance to challenge infections with herpes virus and the murine retrovirus, Friend leukemia virus (33). The mechanisms responsible for these drug effects were not established, although modulation of cytokines such as IFN and modulation of NK activity were suggested (9, 33).

THC and *Legionella pneumophila* Infection

Recent studies in this laboratory have been concerned with the effects of THC on infection with *Legionella pneumophila* (Lp). This organism causes Legionnaires' disease and when it first gained notoriety in 1976 was feared to represent a serious threat to humans. However, subsequent laboratory and environmental studies demonstrated that Lp was not only susceptible to treatment with antibiotics but also was widely distributed in the environment resulting in frequent exposure and therefore low levels of immunity in both humans and animals. Lp is an intracellular, bacterial pathogen that preferentially infects and replicates in host cells such as macrophages. This intracellular life cycle is shared with other medically im-

portant bacterial pathogens such as the tuberculosis bacillus and therefore studies aimed at examining the effects of marijuana on Lp infection have a broader application to infections caused by this group of intracellular bacterial pathogens.

Resistance to and recovery from infection with Lp appears to depend primarily on the activation of killer cells and generation of protective cytokines. The role of specific antibodies in protection is uncertain, in spite of the fact that the first immune response documented in humans was an increase in specific antibodies (34). Immunized and infected guinea pigs were shown to have high levels of serum antibody (35, 36), but these antibodies appeared to promote the uptake of the bacteria by mononuclear cells rather than inhibit infection (37). In addition to antibodies, humans (38, 39), guinea pigs (35, 40–42), and other animals (43, 44) display many manifestations of cell mediated immunity (CMI) when exposed to Lp antigens. These include enhanced lymphocyte proliferation (39, 42), macrophage activation (38, 45), cytokine production (46–50), natural killer cell activity, which can result in lysis of Lp-infected monocytes (51), and mobilization and activation of neutrophils (47). Many of these effects are probably due to an infection-induced mobilization of various cytokines, since cytokines are known to regulate the above manifestations of CMI and cytokines can reconstitute resistance to Lp infection in immunosuppressed animals (52). Because of the importance of cytokines in resistance of mice to Lp infection, it seemed likely that this infection model would be suitable for studying the interaction of THC, cytokines, and infection.

Animal infection models can be readily used to study the immune aspects of both primary and secondary infection. The Lp model, therefore, was used to study the effects of THC injection on both types of infection. BALB/c mice easily survive a primary infection with an Lp dose of 7×10^6 cfu per animal and experiments were performed to determine whether relatively low doses of THC (1–5 mg/kg/day), either the day before or the day after infection, increased host susceptibility to the primary infection. Mortality of the mice was not increased by drug treatment under these conditions (53). Animals given a sublethal, primary infection frequently develop secondary immunity to a subsequent infection with the same microbe administered several weeks later. This secondary immune response is very important for host survival and is the basis for vaccine prophylaxis. Therefore, to test the effect of THC on secondary immunity, the Lp-primed mice were challenged with a secondary lethal dose (e.g., 5×10^7 cfu) of Lp. Mice treated with a primary infection survived the secondary challenge infection. However, mortality after challenge infection was significantly increased in mice that had been drug treated and primed with Lp 3 weeks earlier, indicating that THC injection at the time of primary infection suppressed development of secondary immunity (54).

Since marijuana is usually taken more than once, experiments were also performed to examine resistance to infection in mice treated with two doses of THC. In the dose range of 1–5 mg/kg/day given 1 day before and 1 day after primary infection, no effects on survival were observed, although morbidity (e.g., malaise, etc.) appeared greater than in animals given only 1 drug injection. However, by increasing the drug dose to 8 mg/kg/day given 1 day before and 1 day after primary infection, mortality increased dramatically beginning as soon as 30 min following the second THC injection (55).

It is concluded from the above observations that THC injection into mice significantly modifies the course of both primary and secondary infection with Lp. As already mentioned, Lp infection induces mobilization of cytokines. From the shock-like death observed following THC treatment and primary infection, it appeared that administration of THC coincident with infection mobilized cytokines to toxic levels. It is known that shock and collapse secondary to infection are due to acute phase cytokines, and indeed it was found that levels of these cytokines (e.g., TNF-α and IL-6) increased in the serum of the Lp infected and THC injected mice. In addition, administration of monoclonal antibodies to either TNF-α, IL-6 or IL-1α and -β protected these mice from the drug induced mortality (55). The mechanisms of the mobilization of toxic levels of cytokines is not clear at this time. However, changes in arachidonic acid metabolites might be involved because the production of these metabolites and cytokines are closely linked (56) and THC treatment has been associated with changes in arachidonic acid metabolism (57).

As shown above, THC also suppressed the development of secondary immunity to Lp infection (54). Immunity to Lp is dependent upon the development of CMI and the level of CMI is regulated by the level of T helper cells. Two subsets of T helper cells have been defined based upon the cytokine profiles of T cell clones (58). These subsets, termed Th1 and Th2, have contrasting roles in the immune response (58, 59). For example, Th1 cells produce IL-2 and IFN-γ and are primarily involved in CMI, while Th2 cells produce IL-4, IL-5, IL-6 and IL-10 and are primarily involved with humoral immunity. In addition, the two T cell subsets appear to regulate the production of different IgG isotypes by B cells (60). Th1 cells stimulate more IgG_{2a} antibodies while Th2 cells stimulate more IgG_1 antibody. Several studies have recently demonstrated the importance of Th1 cell-derived cytokines in CMI

resistance to bacteria (61, 62). With this in mind, experiments were performed to determine whether mice infected with Lp and administered THC showed alteration in the relative activities of Th1 and Th2 cells (63). Mice were either infected with Lp only or infected and injected with THC, and the blood serum and splenocytes removed for study. Cultured splenocytes from Lp-infected mice developed an expected vigorous lymphoproliferation when stimulated with Lp antigens. The response was highest 1 week after infection. However, spleen cells from mice injected with THC and infected with Lp showed significantly reduced antigen-specific proliferation, suggesting a drug-induced attenuation of the CMI response. Mice sublethally infected with Lp develop serum antibodies specific for Lp. Using an enzyme-linked immunosorbent assay (ELISA) to analyze these antibodies, it was found that anti-Lp antibody were detected in both THC-treated and nontreated Lp-infected mice (63). However, when antibodies of the IgG_{2a} or IgG_1 isotypes were measured by ELISA, increased production of IgG_1 was observed in the drug-treated mice. These results indicated the possibility of a shift in the relative activities of Th1 and Th2 cells in drug treated mice.

Changes in Th1 and Th2 activities occurring *in vivo* can be demonstrated *in vitro* by removing lymphocytes from test animals, stimulating the cells in culture with a mitogen, and measuring the relative production of either Th1 cytokines (e.g., IFN-γ) or Th2 cytokines (e.g., IL-4). Therefore, mice infected with Lp and also treated with THC were sacrificed, spleen cells obtained, stimulated in culture with mitogen for 24 hr, and the supernatants assayed by ELISA for IFN-γ protein. It was found (63) that splenocytes from THC-treated and infected mice were deficient in IFN-γ production compared with spleen cells from animals that were infected only. This supported the view that there was indeed a shift from Th1 to Th2 subtypes in the spleen of THC-treated animals. Additional studies showed THC treatment of cultured, normal splenocytes directly induced a shift from Th1 to Th2 activity in that the drug- and mitogen-treated cultures produced more IL-4 and less IFN-γ compared with mitogen-only treated cultures (63). As with drug effects on acute phase cytokine mobilization noted above, the exact mechanism for the drug-induced shift in the balance of Th1 and Th2 cells is not known at this time. However, the balance of these two cells is controlled by the levels of cytokines such as IL-1 (64) and IFN-γ (65), and THC treatment has been reported to increase the former (12) but decrease the latter (7). Studies are currently in progress to define more thoroughly the cytokine and lymphocyte profiles in drug-treated and infected animals to understand the mechanism of decreased CMI and consequent enhanced susceptibility to infection.

Conclusions

It is clear THC, the major psychoactive cannabinoid in marijuana, modulates the function of immune cells, including the production and/or release of cytokines. Furthermore, it is clear drug effects on immune cells and cytokines can be demonstrated in animal models of infection with agents such as Lp. In light of the fact that cells of the immune system are now believed to express cannabinoid receptors, it will be of interest to establish the role of these receptors in drug effects on host immune mechanisms as well as the role of these receptors in the normal functioning of the immune response.

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