REVIEW ARTICLE

Immune System and Methamphetamine: Molecular Basis of a Relationship

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Abstract: The use of methamphetamine (Meth) as a drug of abuse is on the rise worldwide. Besides its effect on the function of the brain, Meth has detrimental effects on how the immune system functions. As documented in the literature, various experimental models (cellular, animal, mice, and non-human primates) have been used that have contributed to the overall knowledge about immune system impairments from Meth exposure. It has to be noted that while Meth is used in very few treatments, it affects a broad range of biological mechanisms, not only immune regulation, in a negative manner. Undoubtfully, the effect of Meth is highly complex; moreover, the initial molecular triggers remain unknown. The analyses of available literature suggest that the effect of Meth is not prompted by one underlying mechanism. Although the effect of Meth might be either acute or long-lasting, the overall effect is negative. Further advancement of our knowledge on Meth's specific actions will require systematic experimental approaches using all available models. In addition, bioinformatic analyses are necessary to build a comprehensive model as a needed tool to fill the gap in knowledge.

1. INTRODUCTION

Besides damage to the central nervous system (CNS), methamphetamine (Meth) has detrimental effects on the immune system. As expected, the vast majority of studies published to date are focused on the concurrent effect of HIV-1 infection and Meth use. The use of Meth is also associated with other infections (*e.g.*, MRSA (methicillin-resistant staphylococcus aureus) skin and soft tissue infections) [1], TB (tuberculosis) [2], and urinary tract infections [3]. Meth also enhances *Cryptococcus neoformans* pulmonary infection and its dissemination to the brain [4]. All of this indicates that Meth use impairs the immune system, challenging its defense against these infections.

A mounting body of experimental evidence indicates that there is increased inflammation in the immune system when it is exposed to Meth [5, 6]. Despite increasing studies, the exact mechanisms of pro-inflammatory effects are not clear. Multiple studies propose that Meth use can modulate the immune response *via* molecular mechanisms [6-8]. One study by Liu and his colleagues postulates that Alpha 7 nicotinic acetylcholine receptor (α7 nAChR) is required for the modulation of an inflammatory response through the cholinergic pathway [9]. This has been further supported by

Maldifassi *et. al* [10], showing that dupalpha 7 subunit (*dupα 7*) gene expression levels in peripheral blood mononuclear cells (PBMCs) correlates with serum concentrations of inflammatory biomarkers. However, this study was performed using RAW264.7 mice macrophages and needs further investigation in models that are more closely related to the *in vivo* situation in humans (Fig. **1**).

It is not clear whether observed effects of Meth exposure are directly or indirectly associated with other cells that are not included in the immune system (*e.g.*, astrocytes). For example, interleukin-15 (IL-15) is upregulated in astrocytes inducing the proliferation of T cells feeding into inflammatory responses of the innate immune cells in the brain [11]. However, it has to be emphasized that CNS inflammatory responses might be governed by different triggers than responses of the immune system on the periphery [12]. As it is known, the brain is "hidden" behind or protected by the blood-brain barrier (BBB); therefore, cells of the immune system, particularly those that do not proliferate, need to get a signal to migrate into the CNS. Moreover, neuroinflammation caused by drugs of abuse is mostly considered in the context of human immunodeficiency virus-1 (HIV-1) infection. This is associated with the fact that Meth use and other drugs of abuse leads to needle sharing and inevitably to hepatitis C (HCV) and HIV-1 infections [13]. Another effect, as postulated by Gaskill *et al*., is an increase of dopamine in the CNS that may impact the functions of monocytes, macrophages, and T cells, including an increase of HIV-1 infection [14]. In many instances, HIV-1 infection of the brain leads to

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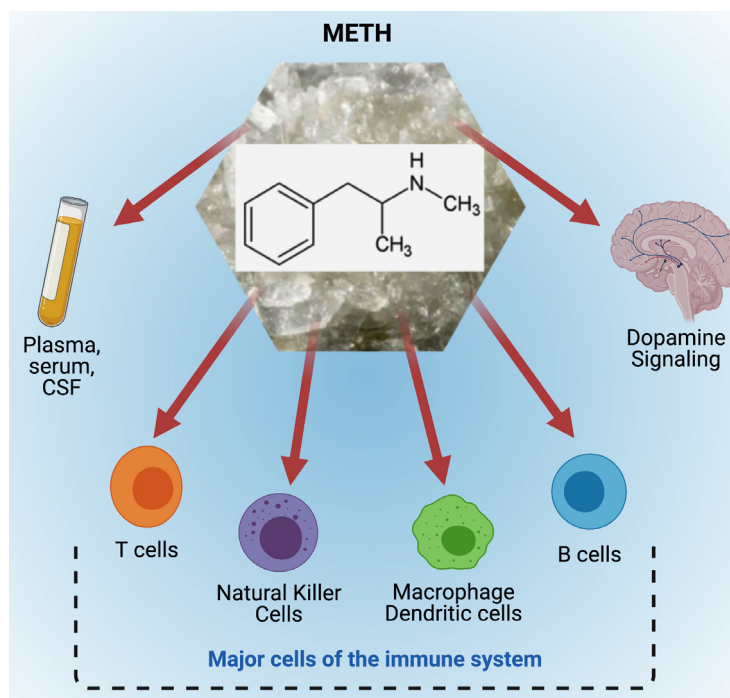E-mail: pciborowski@unmc.edu

Fig. (1). Besides affecting brain functions through the dopaminergic system [24], Meth, as well as other psychostimulants, have detrimental effects on the immune system. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

HIV-associated neurocognitive disorders (HAND), and concurrent use of Meth augments cognitive impairments. The molecular mechanism of this pathology is poorly understood as it is multifaceted and involves disorders of the BBB and the action of neurotoxins, such as envelope glycoprotein gp120, Tat, and Nef HIV proteins [9].

One question that remains to be addressed is whether drugs of abuse, such as cocaine, Meth, oxycodone (OxyContin), *etc*., have one underlying molecular effect or do each of them, although not profound, have differences in how they affect the immune system. Reynolds *et al*. showed the effect of cocaine or Meth on the expression of six proteins, three in each group, through the Western blot analysis [8]. None of the proteins in each group overlaps, suggesting that the effect of cocaine leads to different proteomic outcomes than Meth, further leading to the conclusion of some differences in the molecular mechanisms underlying conditions, such as HCV and HIV-1.

Based on the results from several experimental models, the association of Meth enhancing HIV-1 infection became evident [15-17]. Quite a few questions have been asked, such as whether Meth affects primary targets of HIV-1 infection, T cells, and macrophages. The dominant notion is that Meth enhances HIV-1 replication [18]. Toussi *et al*. proposed a mechanism in which Meth activates transcription of the HIV long terminal repeat (LTR) regulatory region in association with translocation of Nuclear factor NF-κ-B (NFκB), thereby demonstrating that Meth accelerates disease course in HIV-infected individuals [18]. Lawson *et al*. investigated the effects of Meth on the role of miRNA (microRNA)-146a and postulates that Meth induces miRNA-146a and triggers an interleukin-1β (IL-1β) auto-regulatory loop that modifies innate immune signaling in $CD4^+$ T-cells [19]. Furthermore,

Mantri and his colleagues postulated that Meth causes up-regulation of anti-HIV-1 miRNA-125b, miRNA-150, and miRNA-28-5p RNAs in CD+ T cells [20]. Another study suggests that miRNAs play an important role in fine-tuning alterations of the innate immune system [19, 21]. These latter studies clearly indicate the complexity of Meth use and HIV-1 pathogenicity.

Taken together, the role of miRNAs seems to be evident; nevertheless, it is still not clear whether these effects are primary or secondary triggers. Higher viral loads and lower CD4+ T cell counts in HIV-infected individuals using Meth have also been reported and linked to the activation of innate immunity, mediated by Toll-like receptor 9 (TLR-9) and interferon-γ (INF-γ) [22]. The expression of pro-inflammatory cytokines [7, 23], such as interleukin-1β (IL-1β), has been shown in many studies to be associated with HIV-1 infection [19]. Markedly, the full effect of Meth abuse on miRNA expression remains unknown.

In summary, the effect of Meth on the function of the immune system is understudied. The studies of the pharmacokinetics of Meth in the human body were focused mostly on the detection of Meth and its metabolites in blood. More is known about the metabolism of Meth in the CNS, however, not much is known about other tissues. More information can be gathered from review publications, such as one published by Courtney and Ray [25]. So far, it has not been shown that there is a link between addiction *per se* and pro-inflammatory responses of the immune system. It seems that persons with drug addiction have a higher susceptibility to various infections, which is secondary to addiction and may not be apparent at the early stages of drug use. A proteomic study performed by Pottiez *et al*. indicates that plasma proteome returns to levels of non-users after a relatively short

abstinence [26]. However, this proteomic study did not reveal profound and long-lasting effects, which might still exist in much milder forms. Nevertheless, this aspect of human health is equally important from the perspective of treatment and health management.

2. HOW LONG DOES METH PERSIST IN TISSUES? PHARMACOKINETIC CONSIDERATIONS

Meth is a potent neuro-stimulant that triggers a release of catecholamines, such as norepinephrine, dopamine, and serotonin, as well as inhinits their reuptake in the brain. The psychological and physiological outcomes of these molecular events resemble the epinephrine-induced so-called fight-or-flight reaction of the body. Some features of this reaction, such as loss of appetite, have been observed when Meth tablets are prescribed for exogenous obesity treatment. Meth also showed benefits in ADHD (attention deficit hyperactivity disorder) treatment, although the mechanism of action in this case also remains unknown [27]. After oral administration, Meth is rapidly absorbed from the gastrointestinal tract to the blood and reaches the highest concentrations between 3.13-6.3 hours post-ingestion. However, due to other effects, such as euphoria, hallucinations, enhanced alertness, or fatigue elimination, Meth is predominantly used as an illicit substance. In such cases, Meth is often self-administered by drug abusers through different routes, *e.g.*, intra-venous (i.v.) injection or inhaled through smoking to achieve a faster and more potent psychostimulant effect. Meth metabolite, (+)-amphetamine, also show psychostimulant properties [28]. A systematic review of Meth levels in body fluids after various routes of use has been provided by Cruickshank and Dyer [29]. It is important to note that data provided in this excellent review include plasma, urine, and oral fluid but not solid tissues or cerebrospinal fluid (CSF). For investigations on Meth pharmacokinetics in solid tissue, animal models are used, such as one presented by Cho *et al.* [30]. This information can be used with some approximation to evaluate how much and for how long cells of the immune M Methμsystem are exposed to Meth. In our cell-based experiments, we used 100 [7]. Although higher concentrations were used by others, we observed that at higher concentrations, Meth caused cytotoxicity in *ex vivo* experiments using primary macrophages [7, 31].

The pharmacokinetic characteristics of Meth are important for the evaluation and understanding of the mechanisms by which it triggers different effects on the body. Riviére *et al.* investigated the pharmacokinetics of *i.v.* use [28]. Meth injection in rats found that (+)-amphetamine makes up approximately one-third of the drug in tissue. The authors demonstrated the importance of both molecules in triggering pharmacological effects following *i.v.* administration of Meth. They showed that after *i.v.* injection, Meth, and (+)-amphetamine are rapidly distributed to different tissues and organs. Meth reached the peak concentration in all investigated tissues just after injection (at 2 min, the first measured point), except the spleen, where the highest levels were observed 10 min after injection. In contrast, (+)-amphetamine, showed the highest levels in blood 20 min after Meth injection, and its elimination half-life in serum was calculated to be 74.9 min., compared to 54.2 min for

(+)-Meth. The brain-to-serum Meth levels reached the highest point of 13:120 min after injection. Meth accumulated mainly in kidneys, less in the spleen, brain, liver, heart, and least in serum. Whole-body distribution and bioavailability of Meth after injection was also examined by Volkow and her colleagues on the group of healthy human study participants [32]. They used Positron Emission Tomography (PET) and [^{11}C]d-methamphetamine to measure the drug peak uptake (% Dose/cc), rate of clearance (time to reach 50% peak-clearance), and accumulation (area under the curve) in different organs. The [^{11}C]d-methamphetamine peak concentration was the highest in kidneys and lungs and further decreased in stomach, pancreas, spleen, liver, and finally in heart and brain, where it was the lowest. The highest uptake of the Meth was observed in the lungs and liver (22% and 23% of the dose, respectively), while brain and kidneys were characterized by intermediate Meth uptake levels (10% and 7% of the dose, respectively). However, in terms of the speed of the Meth uptake, it ranged from 55-60 sec in lung and heart, 3-5 min in spleen, kidneys, and pancreas, 9 min in the brain, and up to 30 min in stomach and liver. The fastest Meth clearance was observed in the lungs and heart (7-16 min), whereas, in the kidneys, spleen, and pancreas, it was in an intermediate range (22-50 min). In turn, Meth clearance was the slowest in the brain, liver, and stomach, wherein it exceeded 75 min.

3. EXPERIMENTAL MODELS

The investigation of the effect of Meth on the complex immune system requires various approaches. The experimental models need to cover and combine results from *in vitro*, *ex vivo,* and *in vivo* studies. Here, we briefly review three groups of models: cellular (*in vitro* and *ex vivo*), rodent (*in vivo*), and non-human primates (*in vivo*, *ex vivo*) and how much each model helps in advancing our understanding of Meth effects. One study used *Drosophila melanogaster* as an experimental model to investigate oxidative stress and carbohydrate metabolism induced by Meth treatment [33]. Each model has strengths related to the questions asked.

3.1. Cellular Models

Cellular models are indispensable and widely used for a variety of studies of the cellular processes, including effects of Meth as well as the combination of Meth exposure and co-morbid infections. These models allow studies of the metabolic pathways and define alterations in every step. One drawback of using an isolated and pure population of cells is that they are in isolation from their natural environment. Therefore, results from cellular studies need to be validated using other systems reflecting closer complexity of *in vivo* environment. Recently, an organoid technology has been proposed as a middle step between cellular and animal models [34].

There are two groups of cellular models; one consists of a number of transformed cell lines, including THP-1 cells, Jurkat cells, *etc*. A second cellular model comprises primary cells, T cells, monocyte-derived macrophages (MDM), dendritic cells, natural killer (NK) cells, *etc*. Cells might be of human origin as well as derived from various animals, *i.e.*, rodents or non-human primates.

Besides human cell lines, RAW 264.7 cells, which are a macrophage-like transformed cell line derived from BALB/c mice, have been widely used in a vast array of cell biology, immunology, and oncology studies. However, the use of this cell model in studying the effects of Meth has been limited [35].

3.2. Rodents

Rodents, specifically mice, are widely used to study various aspects of the immune system. They were also being used to study the effects of Meth. As shown by Yu and his colleagues, upon chronic Meth exposure, mice splenocytes significantly decreased the production of interleukin-2 (IL-2), but did not affect the production of interleukin-4 (IL-4) and interleukin-6 (IL-6) [36]. However, the authors observed a significant increase of tumor necrosis factor- α (TNF-α). The overall conclusion from this study was that Meth suppressed T helper type 1 cells (Th1) and enhanced T helper type 2 cells (Th2) cytokine secretion. Another study using mice exposed to Meth also showed that splenocytes produced less IL-4 and IFN-γ upon *ex vivo* restimulation with ovalbumin than control [37]. In addition, this study showed attenuated production of ovalbumin-specific IgM, IgG$_1$, and IgG$_{2a}$ antibodies. The unchanged levels of TNF-α were reported by Mata *et al.* in a rat model of the Meth effect [38]. In this study, serum levels of INF- γ, and IL-6 were unchanged. In addition, Meth did not alter the frequency of TNF-α-producing CD4+ T cells; however, it led to a higher frequency of CD8+ T cells, though fewer them produced TNF-α. Increased or decreased expression of mentioned cytokines evidently showed that cells of the immune system are affected, and the overall effect is a pro-inflammatory environment. In addition, alterations in expression of IL-2 and IL-6 show that more than one type of immune cells is affected. Considering that cytokines are triggered by various stimuli and that they regulate responses of other cells of this system, for example activation of B cells by IL-4, it is difficult to dissect primary and secondary factors in the overall effect of exposure to Meth or other drugs. Therefore, building a comprehensive model is complicated and in order to be successful, algorithms that consider multiple variables are required.

3.3. Non-human Primates

Non-human primates represent a model most closely representing *in vivo* in humans. A major advantage of this model is that it can be controlled with a specific time and dose of Meth administration; diet and overall health assessment of the animals are also well controlled. Although it does not remove all variabilities, this model provides more consistent results. Non-human primates as an animal model have been used mostly in the context of simian immunodeficiency virus (SIV) infection, mimicking HIV-1 infection in humans [39-42]. A recent study by Niu *et al.* using monkeys showed Meth increased SIV infection. In addition, the authors showed that Meth affected infected as well as uninfected microglia and brain macrophages. These data provide additional evidence that the effect of drugs of abuse is a very complex pathology. We can extrapolate results from this study using non-human primates to investigate the pathology in the CNS of humans infected with HIV-1 and exposed to drugs abuse [41]. Undeniably, results from studies using non-human primates confirm the pro-inflammatory effect of Meth observed in many studies done with *ex vivo* cells and body fluids of human subjects [11, 43, 44]. The results from these studies provide directions how future studies should be performed using approved clinical material from human subjects.

4. T CELLS AND METH

Among all cells constituting the immune system, the effect of Meth on T cells has been studied most extensively. Across all models and experimental conditions, it has been shown that Meth has detrimental effects on T cells. The vast majority of reports focus on the concurrent effects of Meth and HIV-1 infection and much less on other infections [18]. These studies show that this effect is complex and multifaceted, and their authors proposed numerous potential mechanisms. Multiple studies have shown a connection between cytokine release and miRNAs as a major factor involved in mediating the effect of Meth on T cells and the role of this effect on HIV-1 replication. Meth augments HIV-1 replication in CD4+ T cells and operates through the upregulation of miRNA-34c-5p as an activator. However, this effect is multifaceted, and several CD4+ T cells' activators are induced [45]. The authors also showed that Meth mediated activation of T cells resulted in the activation of several transcription factors, such as NFκB, cyclic AMP-responsive element-binding protein (CREB), and nuclear factor of activated T-cells 1 (NFAT1). This is in line with many studies showing a complex contribution of Meth on increased HIV-1 replication and production of inflammatory cytokines. Lawson *et al.* showed that Meth initiates an interleukin-1 β (IL-1β) feedback loop to alter innate immune pathways that favors HIV-1 replication in T cells [19].

Therefore, cytokine production and release seem to be the best measure of describing the state of suppression and/or activation of CD4+ T cells as well as macrophages and microglia in the CNS and periphery [46]. Meth also leads to the impairment of proliferation of T cells stimulated by concanavalin A (Con A) or lipopolysaccharide (LPS) after two weeks of Meth exposure in mice [27].

Increasing data support the notion that Meth induces oxidative stress. Each study investigating this mechanism showed different aspects of this perturbation ranging from mitochondrial dysfunction [47] to T cells [48]. The latter study postulated that Meth exposure causes increased levels of intracellular calcium and increased levels of reactive oxygen species (ROS). Meth administration leads to mitochondrial dysfunction by decreasing mitochondrial membrane potential and tapering levels of several complexes in the electron transport chain. As Potula *et al.* demonstrated that generated ROS led to a redox-dependent pathway, facilitating T cell immune impairment [48]. As much as such effect is strongly supported by experimental evidence, the initial trigger caused by Meth remains elusive.

5. MACROPHAGE AND METH

Mounting evidence shows that Meth leads to phenotypic alterations and changes in functionality associated with an

increase of macrophage proinflammatory responses. Several studies showed consistently that macrophages and dendritic cells respond to Meth exposure in a dose-dependent manner [7, 49, 50]. It has to be noted that a Meth concentration above 250 mM causes increase in the cytotoxicity, shadowing clarity of results interpretation. A linear increase of response to Meth exposure ranging from 1 to 250 mM and lack of a plateau may suggest that Meth initiates changes in macrophage *via* multiple mechanisms; however, a specific receptor, if such exists, is not a predominant trigger. One can hypothesize that activation of macrophages and dendritic cells can be a sum of effects in multiple intracellular compartments turned on in a sequential manner. There is also consensus that Meth leads to the induction of a proinflammatory phenotype in macrophages, although the mechanism of such response is not known. Also, there is no known or well-defined receptor that might be responsible for transmitting signals leading to macrophage activation and proinflammatory responses. It can be hypothesized that the activation of macrophages and dendritic cells can be a sum of effects in multiple intracellular compartments. Another hypothesis is that Meth will penetrate macrophages and cause acidification of the cells' interior or at least some intracellular compartments, leading to metabolic and signaling changes.

Dendritic cells are potent players in orchestrating T cell immune responses, originated from progenitor cells through hematopoiesis. Bone marrow retains relatively high levels of Meth due to its lipophilic environment and may have a profound impact on hematopoiesis. As expected, Meth regulates the expression of several proteins, including CXCR3, protein disulfide isomerase, procathepsin B, peroxiredoxin, and galectin-1 [49, 51]. This observation is in line with a report published by Harms *et al.* that showed that, in a mouse model, Meth exposure leads to specific phenotypic changes and subsequently leads to changes of functions of T cells as well as other subsets of cells constituting the immune system. More specifically, Meth exposure reduces the overall abundance of activated/antigen-experienced CD4 and CD8 T cells while promoting activation and expansion of discrete CD4 T cell subsets [50]. As reported by Potula *et al.*, the observed changes include reduced IL-2 secretion and T cell proliferative responses after TCR-CD28 stimulation, indicating impaired T cell function [48]. This is in sync with other studies and can serve as novel observations as well as validation.

6. NATURAL KILLER CELLS AND METH

Natural killer (NK) cells detect the major histocompatibility complex (MHC) presented on the virally infected cell surface, generating cytokine release and causing the death of the infected cells. Thus, they are critical to the innate immune system and are similar to cytotoxic T cells in the adaptive immune response.

By using a mouse model, Harms *et al.* showed that Meth administration led to a decrease in the abundance of NK cells, and those remaining had a phenotype, suggesting reduced responsiveness and effectiveness [50]. Interestingly, amphetamine suppressed to some extent the NK cell function while Meth enhanced these functions. At the same time, the authors did not detect an effect of cathinone, which is similar to amphetamines. Taken this together, one can conclude that each of these compounds initiate metabolic effects using different triggers. This remains a hypothesis and needs to be formally tested. Such low numbers of NK cells with a higher proportion of less responsive ones may negatively interfere with the induction of proper immune responses. This is especially important in cases of viral infections, for example, in HIV, where the decrease of NK cell activity was shown to correlate with symptoms [52]. Thus, additional decreased levels and functions of NK cells may further contribute to the susceptibility of viral infections in Meth users.

7. B CELLS

The effect of Meth on B cells has been the least studied among all subsets of cells constituting the immune system. Nonetheless, results from already published studies showed that Meth influences some functions of these cells. For example, mouse B cells exposed to Meth showed impaired proliferative response to mitogens, such as Con A or LPS. Interestingly, this effect was present after 14 days of exposure but not visible after seven days of exposure [27]. Other studies, as discussed in this review, indicate that the effect of Meth on cytokine release is rapid and rather short-lasting [7].

8. HIGH THROUGHPUT STUDIES

High throughput studies are essential for getting a broader view of how the complex biological system works. Such studies provide enormous amounts of data that need to be validated on a large scale before they are analyzed. Besides ontology or PANTHER [53] types of analyses showing affected processes, further bioinformatic investigations are needed. Proteomics studies may show little diversity of responses within the population of cells used for experiments if differences are subtle or sub-populations affected by treatment are very small. Immunohistostaining, whether tissue sections or cells in culture, shows such diversity of responses, and in many cases, they are much greater than anticipated. Flow cytometry analyses show diversity in the population of cells; however, it is limited to how many different proteins can be tracked at the same time. Mass cytometry (CyTOF) analysis can expand the number of proteins measured during one analytical experiment; a few studies used this platform for neuronal rather than immune cell investigations. A new approach leads research to a single-cell analysis followed by visualization of multidimensional sets of data. Uniform Manifold Approximation and Projection (UMAP) is a graph algorithm to arrange data in low-dimensional space. Niu *et al.* used this algorithm to show the effect of Meth on CD11b positive cells isolated from brains of control and Meth treated *Rhesus macaques* [41]. All monkeys were SIV+. The authors concluded that Meth treated animals overexpressed genes involved in morbidity, cell death, and deficiencies in the brain-derived neurotrophic factor (BDNF)-signaling pathways. As expected, the neuroinflammation signaling pathway was, upregulated in SIV-infected animals, while cells from SIV+ and Meth showed clustering cells with enriched glycolysis and Pentose Phosphate Pathway. The authors also proposed that the BDNF pathway might be a target for future treatment since this pathway was inhibited by Meth.

8.1. Genomics in the Evaluation of Meth's Influence on Brain Microglia Cells

Genomics technology has been used to profile changes induced by Meth treated and/or HIV-1 infection in cells of the immune system. Najera *et al*. studied the influence of Meth abuse on gene expression of brain-derived microglia on SIV-infected macaques, a model of NeuroAIDS [44]. By using gene microarray technology, they found that Meth alone strongly affects immune pathways-associated genes, in particular those related to inflammation and chemotaxis. A strong correlation was found between Meth exposure and an increase of chemokine and chemokine receptor-associated molecules levels, such as CXCR4 (C-X-C chemokine receptor type 4) and CCR5 (C-C chemokine receptor type 5). The authors confirmed that Meth-induced upregulation of CCR5 expression in the brain correlated with an increase in viral levels.

8.2. Meth in Body Fluids: Proteomics Approach

We would expect that the use of Meth would show changes in the proteome of blood/plasma, CSF, and other body fluids. These studies are summarized in Table **1**. Nevertheless, most research on the levels of Meth in body fluids was focused on forensic type measurements, answering the question of whether blood samples contain traces of this drug of abuse or not [54, 55]. Understandably, the second major avenue of studies is the effect of Meth on the CNS and/or behavior, also studied in animal models [56]. On the contrary, the relation of the presence of Meth as well as other drugs of abuse in blood and their effects on the function of the immune system has been understudied. Although there is agreement that Meth has the ability to modulate cells of the immune system, there is less of a consensus on how long-lasting such effects are. We would expect that the blood/plasma/serum proteome should provide needed insight information about the functions of the immune system exposed to the toxic effect of Meth.

The experimental data from the few systematic studies of the body fluids' proteome limits answers to the effect of Meth use. One systematic proteomic study performed by Pottiez *et al*. measured changes in plasma samples from users who withdrew from using Meth. This study revealed few changes in the levels of several proteins for a time longer than several weeks [26]. Most notably, among 390 proteins identified, 28 showed significant changes in the expression in the HIV+/persistent Meth+ group over the two visits, which were not attributable to HIV itself. These proteins were involved in complement, coagulation pathways, and oxidative stress. The authors showed quantitative changes in the expression of ceruloplasmin, vitamin D-binding protein, and plasminogen in the group, which measures changes in the proteome of HIV-infected individuals when Meth was still used. To summarize, we can conclude that the use of Meth is reflected by changes in plasma proteomes; nevertheless, these changes are shorter than expected after the use of Meth has been ceased. Importantly, this proteomic study reinforced the opinion that Meth leads to oxidative stress, not only in neurons but also in other types of cells. By using two-dimensional gel electrophoresis (2-DE), Shi *et al*. showed upregulation of Factor H in plasma of Meth addicts

[57]. By increasing Factor H, which is one of the complement activators, the body's response to toxic substances is presented; nevertheless, Factor H is also upregulated in many other pathologies and remains significant but not specific for Meth use.

Some proteome work has been done using biological material from animal models, such as non-human primates [40]. This study showed a significant increase of four proteins in plasma samples of *Rhesus macaques* infected with SIV and treated with Meth. These proteins-IgG lambda chain, extracellular superoxide dismutase, complement factor I, and Mannan-binding lectin serine protease 2-might be evidence of increased inflammatory responses due to the presence of Meth. While being important indicators, these proteins are not specific to Meth exposure during SIV infection.

As much as the effect of Meth on cerebrospinal fluid has been studied to some extent, all of these studies are focused on a specific factor and/or pathway, *i.e.*, oxidative stress in HIV-1 positive Meth users [58] or DRD2/ANKK1 in CSF of Meth intoxicated individuals [59]. As of this writing, systematic proteomic profiling of CSF from individuals using Meth has not been done. Nevertheless, it is important to note that the effect of Meth on oxidative stress has been present in the CSF of human subjects using Meth.

Another systematic proteomic profiling of blood samples from SIV infected and/or opiate treated *Rhesus macaques* was published by Wiederin *et al*. [42]. This iTRAQ (isobaric tags for relative and absolute quantitation labeling) based proteomic study showed changes in blood levels of several proteins. As shown in Table **1**, two separate studies of proteomic profiling of plasma from monkeys and humans showed significant expression of ceruloplasmin. Although this study was performed with samples from monkeys treated with opiate morphine, not Meth, some results show an intertwining connection between the effect of concurrent use of multiple drugs. This does not exclude the notion that each drug uses different triggers; however, it indicates that many affected pathways are affected similarly regardless of the triggers.

8.3. Metabolomics-contribution to Understanding the Effect of Meth on the Immune System

As much as genomics, proteomics, and cellomics are mostly used to dissect molecular mechanisms employing high throughput approaches, metabolomics is mostly used in the discovery of biomarkers. Metabolomic studies allow the identification of pathways that may support the discovery and understanding of indirect biomarkers. Examples are reported by Steuer *et al*. and McClay *et al*., who showed several metabolome-wide significant associations with acute Meth exposure, including compounds, such as lactate, tryptophan, 2-hydroxyglutarate, and succinate associated with energy as well as phosphocholine and ergothioneine associated with repeated exposure to Meth [60, 61]. Metabolites representing energy metabolism, steroid biosynthesis, and amino acid pathways were related to Meth intake. Other candidates, such as linoleic acid and pregnenolone-sulfate changed in the same direction in response to the intake of more than one drug, implying their specificity for Meth.

Table 1.   Proteins differentially expressed in serum/plasma of Meth.

Protein	Proteomics platform	Source
Alpha-1-acid glycoprotein Transthyretin Complement factor H Apolipoprotein L1 Haptoglobin Transthyretin Haptoglobin	2-Dimensional Difference Gel Electrophoresis-(2D DIGE)	human serum [57]
IgG lambda chain, Extracellular superoxide dismutase, complement factor I, and Mannan-binding lectin serine protease 2	Liquid chromatography with tandem mass spectrometry with the use of isobaric tags for relative and absolute quantitation labelling (LC-MS/MS-iTRAQ)	monkey plasma [40]
Ceruloplasmin Vitamin D-Binding Protein Plasminogen	LC-MS/MS-iTRAQ	human plasma [26]
Gelsolin Vitronectin Ceruloplasmin	LC-MS/MS-iTRAQ	monkey plasma [42]
Glutathione (GSH), 4-hydroxynonenal (HNE) gamma-glutamyltransferase (GGT) glutathione peroxidase (GPx)	Targeted study	human CSF [58]

We need to keep in mind that metabolites are made and/or modified by enzymes that are proteins and should be covered by transcriptomics and proteomics. However, this is not always the case. In addition, secretion or accumulation of measurable metabolites can be shifted in time compared to other screening approaches/methods, such as RNA seq.

One systematic metabolomics study of secreted metabolome of human monocyte-derived macrophages (hMDM) was performed by Pawlak *et al.* [31]. In this in-depth targeted analysis, the authors measured 92 metabolites; besides 11 quantitative differences, they showed the presence of stereoisomers of hydroxy methamphetamine. It has to be noted that the authors used the Multiple Reaction Monitoring approach with a predetermined number of specific, by mass to charge ratio *(m/z)* metabolites. The results of an untargeted metabolomic study reported by Adkins *et al*. were not focused on the immune system and were performed in a mouse model. Nevertheless, Adkins *et al*. identified that homocarnosine and 4-Guanidinobutanoate, GABA-specific metabolites contribute to Meth psychomotor sensitization [62].

Recapitulating, as, in the area of proteomics, there are few metabolomic studies published to date of the effect of Meth on the immune system. Moreover, each study uses a different model, making it difficult to interpret the data and their compilation into one comprehensive landscape.

9. METH, CANCER, AND IMMUNOTHERAPY

There are two major avenues for treating cancer. One is pharmacotherapy, and the other one is immunotherapy. In fact, both ways of treatment can be or are applied. The direct effect of Meth on malignant diseases is not a subject of this review. Indirectly, while methamphetamine and/or cocaine modulate cytokines, it may enhance tumor growth [63]. No such mechanism has been directly proposed for the effect of Meth. We may postulate that immunotherapy would be affected by Meth in a similar way as Meth affects the immune system itself, thus, anti-cancer immunotherapy would be affected due to impaired immune cells. Nevertheless, there is a lack of systematic studies in this direction, which prevents us from drawing more conclusions beyond speculation.

10. METH, NEUROTRANSMITTERS, REWARD SYSTEMS, AND IMMUNE SYSTEM

The possible intertwine of the Meth effect(s) with the immune system in the context of neurotransmitters is a very interesting avenue for future studies. Serotonin is a well-known neurotransmitter that plays an important role in the function of the immune system. Studies summarized by Wu *et al*. suggest that many different types of immune cells express the machinery to generate, store, respond to and/or transport serotonin, including T cells, macrophages, and dendritic cells [64]. Serotonin can modulate macrophage polarization which is central for innate immune responses [65]. However, how Meth or other drugs of abuse connect with serotonin- immune system axis has not been studied. Due to a lack of experimental reports, the only information we can gather is through indirect evidence; this topic is awaiting systematic experimental approaches. The same applies to the glutamate system. The most explored effect is the effect of Meth at the junction of dopamine and macrophage systems that we discussed above [14, 43].

CONCLUSION

There is experimental evidence that the use of Meth leads to pro-inflammatory responses; nevertheless, we are far from

drawing more general conclusions that may aid in the treatment and long-term management of people affected by Meth addiction. Two compartments that should be systematically studied are the immune system in the periphery and the CNS. It seems that such a comparison might provide insights into how other cells in the body affect the immune system. After all, the immune system does not operate in a vacuum, as it gets many signals from a variety of directions.

Despite all of the work that has been done, we are quite far from having a comprehensive model of the effect of Meth on the immune system. Studies done so far span through different models and various experimental designs, and in many cases are focused on specific pathways. The results from high throughput and reductionistic studies need to be combined; this can be accomplished by building models with strong bioinformatics support.

Concurrent use of various drugs, alcohol, tobacco, and differences that might be related to age, ethnicity, and gender further complicate our ability to build a comprehensive model dissecting the effects of the only Meth; The answer to the question on how all these insults affect each other at the molecular level needs to be answered. Cellular and animal models show that each insult, when taken separately, including infections and co-infections, contributes to activation of the immune system and subsequent pathologies such as HAND. We also conclude that these triggers might work in different manners [14, 50, 66].

We observe that continuous Meth use is an unstable condition, altering levels of a number of plasma proteins. Some changes might be subtle and undetected by screening methods. This could be caused by the use of other substances concurrently with Meth, such as alcohol, tobacco, and other drugs, such as cocaine. Determining which observed effect is associated with which insult makes biological conclusions more difficult to dissect.

As much as new studies have contributed to our knowledge on the effects of drugs of abuse, specifically Meth, additional investigations are needed. Moreover, such investigations should be conducted in a comprehensive manner to fill gaps that currently exist. One big gap is the lack of bioinformatic that would rectify the causes and consequences of drugs of abuse.

CONSENT FOR PUBLICATION

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