T regulatory cells as a potential therapeutic target in psychosis? Current challenges and future perspectives

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ABSTRACT

Many studies have reported that patients with psychosis, even before drug treatment, have mildly raised levels of blood cytokines relative to healthy controls. In contrast, there is a remarkable scarcity of studies investigating the cellular basis of immune function and cytokine changes in psychosis. The few flow-cytometry studies have been limited to counting the proportion of the major classes of monocyte and lymphocytes without distinguishing their pro- and anti-inflammatory subsets. Moreover, most of the investigations are cross-sectional and conducted with patients on long-term medication. These features make it difficult to eliminate confounding of illness-related changes by lifestyle factors, disease duration, and long exposure to antipsychotics. This article focuses on regulatory T cells (Tregs), cornerstone immune cells that regulate innate and adaptive immune forces and neuro-immune interactions between astrocytes and microglia. Tregs are also implicated in cardio-metabolic disorders that are common comorbidities of psychosis. We have recently proposed that Tregs are hypofunctional (‘h-Tregs’) in psychosis driven by our clinical findings and other independent research. Our h-Treg-glial imbalance hypothesis offers a new account for the co-occurrence of systemic immune dysregulation and mechanisms of psychosis development. This article extends our recent review, the h-Treg hypothesis, to cover new discoveries on Treg-based therapies from pre-clinical findings and their clinical implications. We provide a detailed characterisation of Treg studies in psychosis, identifying important methodological limitations and perspectives for scientific innovation. The outcomes presented in this article reaffirm our proposed h-Treg state in psychosis and reveals emerging preclinical research suggesting the potential benefit of Treg-enhancing therapies. There is a clear need for longitudinal studies conducted with drug-naïve or minimally treated patients using more sophisticated techniques of flow-cytometry, CyTOF expression markers, and in vitro co-culture assays to formally test the suppressive capacity of Tregs. Investment in Treg research offers major potential benefits in targeting emerging immunomodulatory treatment modalities on person-specific immune dysregulations.

1. Introduction

It is widely held that inflammation plays an important role in the pathogenesis of psychosis, based principally on the large number of studies reporting increased circulating concentrations of cytokines and other inflammatory markers (Corsi-Zuelli et al., 2020, 2021; Goldsmith et al., 2016). Meta-analyses show that interleukin-6 (IL-6) stands out as the most consistently raised cytokine in the cerebrospinal fluid (CSF) (Orlovská-Waast et al., 2019) and blood (Goldsmith et al., 2016) of patients relative to healthy controls, including drug-naïve first-episode...
psychosis (FEP) patients (Pillinger et al., 2018; Upthegrove et al., 2014). In remarkable contrast to the extensive cytokine literature, there have been very few studies of multi-parameter flow cytometry to characterise the cellular components of the immune system. Available studies in psychosis consist of cross-sectional investigations conducted mainly in medicated patients, limited in counting the proportion of major classes of monocyte and lymphocytes without distinguishing their pro- and anti-inflammatory subsets (Miller et al., 2013).

In this article, we focus on regulatory T cells (Tregs) to identify fresh ideas for innovation in mechanisms of neuroimmune dysfunction and treatment in psychosis. Tregs regulate immune homeostasis, suppress inflammation, restrain immune responses (Sakaguchi et al., 2008; Shevach, 2009) and, it is increasingly understood, have regulatory interactions with glial cells in the brain (Ito et al., 2019; Pasciuto et al., 2020; Xie et al., 2015). We briefly recapitulate the role of Tregs in the neuroimmune network and summarise findings that stimulated the development of the hypofunctional Treg (h-Treg) hypothesis of psychosis (Corsi-Zuelli and Deakin 2021). Then, we discuss recent discoveries on Treg-based therapies and consider the dynamics between IL-6 and Tregs and treatment implications. The h-Treg hypothesis requires that Tregs from people with psychosis are hypofunctional. In sections 3 and 4, we review the eight available studies in detail, discuss the methodological limitations and inferences that can be drawn. We conclude by summarising the challenges and perspectives for Treg research in psychosis with ideas for the design of future studies. For information on the Junior Key Opinion Leader, see Fig. 1.

2. Tregs: role in the neuroimmune network and therapeutic implications

2.1. Tregs overview

Tregs are the ‘guardians’ of immune homeostasis, critical in maintaining a balanced innate and adaptive immune function and limiting inappropriate inflammation (Sakaguchi et al., 2008). In the central nervous system (CNS), Tregs regulate homeostatic neuroimmune interactions, especially between astrocytes and microglia (Ito et al., 2019; Pasciuto et al., 2020; Xie et al., 2015). FoxP3, a Forkhead family transcription factor, is the master regulator of Tregs, critical for their development and suppressive capacity (Buckner, 2010). (Epi)genetic loss of Treg homeostasis has been implicated in the pathogenesis of autoimmune disorders (Ohkura et al., 2020; Shu et al., 2017) and common metabolic diseases, such as type 2 diabetes, insulin resistance (Arroyo Hornero et al., 2020; Eller et al., 2011; Paolino et al., 2021; Qiao et al., 2016; Zeng and Chi, 2013) and cardiovascular diseases (Albany et al., 2019; Meng et al., 2016), which are also recognised co-morbidities of psychosis. In addition, Tregs play key roles in healthy pregnancy by promoting maternal tolerance to foetus antigens (Teles et al., 2013). Hypofunctional Tregs during pregnancy is associated with predisposition to obstetric complications, including placental insufficiency, higher risk of infection, inflammation, premature birth (Martini et al., 2020; Rowe et al., 2013), which are well implicated in abnormal foetal neural development (al-Haddad et al., 2019) and considered non-specific risk factors for neuropsychiatric disorders, including psychosis (Davies et al., 2020). Due to space restrictions, for more details, please refer to Fig. 2 and our recent review (Corsi-Zuelli and Deakin 2021).

2.2. H-Treg hypothesis of psychosis

The h-Treg hypothesis (Corsi-Zuelli and Deakin 2021) was prompted by recent lines of evidence from our group and others that are not compatible with the prevalent view that microglial inflammation is central to the pathogenesis of schizophrenia. Deakin et al. (2018) found that minocycline, a potent inhibitor of microglial inflammation, was ineffective in recent-onset patients. The findings were followed by meta-analyses of in vivo Positron Emission Tomography (PET) imaging, providing compelling evidence that the translocator protein TSPO (a putative biomarker for inflamed microglia) is not increased but reduced in psychosis (Plavén-Sigray et al., 2018, 2020), as we had observed in several brain regions of antipsychotic-free patients (Conen et al., 2020). We proposed that microglia are driven into a non-inflammatory state by overactivity of TGF-β mediated astroglial restraint (Conen et al., 2020). We discovered this idea was highly compatible with multiple large-scale transcriptome-wide studies that we reviewed; they consistently reported no change or decreased microglial gene expression but increased astroglial expression in schizophrenia (Gandai et al., 2018a, 2018b; Gonzalez-Peñas et al., 2019; Ramaker et al., 2017; Toker et al., 2018).

Treg hypofunction provided the missing link between low-grade systemic inflammation in psychosis and central glial dysregulation. This insight was triggered by the observation that low-dose methotrexate had a significant antipsychotic effect (Chaudhry et al., 2020); low-dose methotrexate is standard of care in autoimmune disorders through its ability to restore Tregs and their control of the immune response (Avdeeva et al., 2020; Gribbs et al., 2015; Peres et al., 2015, 2018a). Recent findings have revealed that Tregs have a close regulatory interaction with astrocytes (topic 2.1), and we deduced that methotrexate’s efficacy might result from restoring impaired Treg control of astrocytic disinhibition in psychosis. Our discoveries were unexpectedly corroborated by the latest Psychiatric Genetics Consortium report, which identified risk variants of genes...
Impaired Treg-glial interaction

CLINICAL SYMPTOMS

CENTRAL
Thymus-derived Treg (Genetically predisposed to low FoxP3)

PERIPHERAL
Peripherally-induced Treg (hypofunctional due to IL-6 and Teff effects)

COMORBIDITIES

Hypofunctional Tregs

Prenatal complications
Autoimmune disorders
Cardiovascular/metabolic disorders
Peripheral low-grade inflammation/infections

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Fig. 2. Loss of Treg-mediated immune homeostasis and increased risk of psychosis

Tregs are the “guardians” of immune homeostasis; they control the balance between the innate and adaptive immune systems, regulate neuron-glial interactions and limit inappropriate inflammation. Middle: There are two main populations of Tregs (light-green cells) based on their developmental origin, thymus-derived (tTregs, pink box) and peripherally-induced (pTregs, blue box). tTregs develop from CD4+ CD62L+ T cells (grey cell), while pTregs are generated extrathymically from naïve CD4+ T-cells (marsala cells). FoxP3, a Forkhead family transcription factor, is the master regulator of Tregs, critical for their development and suppressive capacity. tTregs may be more prone to FoxP3 destabilisation in psychosis due to polygenic risk variants in Symptons of psychosis may arise from aberrant hypofunctional Treg-glial dysregulation. Tregs in the meninges and brain lymphatic system in the disease are associated with the functional capacity of Tregs (FoxP1 and Furin) (Consortium et al., 2020). In the following sections, we briefly discuss novel emergent approaches to enhancing Treg function in neurological and autoimmune disorders and how these are helping to disentangle mechanisms underlying low-grade inflammation and neuroimmune dysfunction that could be relevant to psychosis in the future.

2.3. Tregs control systemic inflammation and neuroimmune dysfunction

There has been increasing interest in recovering the functional capacity of Tregs or transducing competent Tregs to restore systemic immune control and glial dysfunction. For example, in experimental neuroimmune models, depletion of FoxP3-expressing T cells (a transgenic murine model that allows highly specific depletion of Tregs using diphtheria toxin) induces astrogliosis (Ito et al., 2019; Krämer et al., 2019) with activation of brain IL-6/STAT3 signalling that can be restored by adoptive cell transfer (ACT) of competent Tregs or intraventricular administration of products derived from competent brain Tregs (amphiregulin) (Ito et al., 2019). In human research, current clinical trials are testing ACT of competent Tregs in several autoimmune and neuroimmune diseases, such as graft-versus-host disease, type-1 diabetes, Crohn’s disease, amyotrophic lateral sclerosis, multiple sclerosis (Duggleby et al., 2018; Gliwinski et al., 2017), and most recently COVID-19 (Wang et al., 2021).

In experimental autoimmune encephalomyelitis, Krienke et al. (2021) recently designed a BioNTech mRNA vaccine that codes for disease-related autoantigens optimised for systemic delivery to splenic dendritic cells in the absence of costimulatory signals to treat and prevent the disease. Mice immunised with the newly designed mRNA vaccine had systemic activation of antigen-specific Tregs that suppressed disease-promoting autoreactive T-cells and related cytokines (IFN-γ, TNF-α, IL-6, IL-2, IL-17) without causing systemic immune suppression. This reduced brain and spinal cord demyelination. The vaccine benefit was abolished by antibodies that interfered mostly with Treg functioning (mainly CTLA-4 but also PD-1), as shown by flow cytometry and single-cell RNA sequencing. The study contributes to knowledge on Tregs and neuroimmune dysfunction, but the definition of optimal disease-specific autoantigen is a barrier to clinical translation.

Most recently, Treg research and Treg-based therapies have been expanded to neuropsychiatric disorders such as autism and schizophrenia, in animal models. Xu et al. (2021) induced immune activation in pregnant mice by injection of Toxoplasma gondii antigen and reported abnormally reduced social interaction in the adult male offspring associated with a number of brain changes, including overproduction of IL-6 by astrocytes and abnormal hippocampal neuronal connectivity with no evidence of microglial inflammation. They showed that these changes were mediated by systemic T-cell imbalance (low Treg; high Th1, Th17). Remarkably, ACT of Tregs from control mice reversed the behavioural and brain changes, with maternal pathogen-activated Tregs especially effective and capable of infiltrating the brain parenchyma. In another study, You et al. (2020) used amphetamine to induce schizophreni-like behavioural changes in adult mice and considered human umbilical cord-derived mesenchymal stem cells (hUC-MSC) as a Treg-enhancing therapy. The authors showed that a single injection of hUC-MSC reversed aberrant behavioural changes and the systemic pro-inflammatory TNF-α profile. The principal mechanism of hUC-MSC was upregulation of the Treg-related transcription factor FoxP3 mRNA in the blood and mesenteric lymph node. Given that hUC-MSC are usually limited in reaching the brain, the authors assume restoration of peripheral Tregs from a single hUC-MSC infusion as sufficient for long-term reversal of behavioural and cytokine changes. In human research, MSC transplants are being tested in children with autism (Chez et al., 2018; Riordan et al., 2019), Crohn’s disease (Zhang et al., 2018), diabetes (El-Badawy and El-Badri, 2016), chronic ischemic heart disease (He et al., 2020), and recently in COVID-19 (Maeurer et al., 2021).

There are many challenges to translating these lines of treatment to complex multifactorial disorders, such as psychosis. However, the studies are striking examples of the novel and determining role of Tregs in controlling not only systemic inflammation but also disordered glial functions. Most importantly for this review, Xu et al. (2021) revealed for the first time that restoring impaired Treg capacity unexpectedly corrected neuroimmune dysfunction that resembles those of clinical schizophrenia discussed in section 2.2. This raises critical translational questions about the phenotype and functional capacity of Tregs in patients with psychosis, their correlations with clinical symptoms and with core inflammatory markers of the disease such as IL-6, and identification of potentially repurposable drugs with Tregs-boosting effects, discussed in the following sections.

2.4. Treg-IL-6 interplay as a novel mechanistic pathway in psychosis?

The functional implications of consistently raised IL-6 in the blood and CSF of patients with psychosis, including drug-naïve patients, are unclear. IL-6 is a pleiotropic cytokine that can signal via both membrane-bound (classic) and soluble (trans) receptors. The classic-signalling is usually associated with its regenerative and anti-inflammatory properties, whereas trans-signalling is frequently associated with its pro-inflammatory actions (Calabrese and Rose-John, 2014). Mendelian randomisation, which uses genetic variants that regulate the levels/activity of a biomarker, suggests a causal role of genetic variants related to IL-6 trans-signalling in psychosis (Hartwig et al., 2017). This indicates that associations are unlikely to be a consequence of confounders, such as lifestyle factors.

It is possible that increased IL-6 trans-signalling contributes to or sustains a hypofunctional Treg state in psychosis (Fig. 3). Extensive preclinical and clinical research in autoimmune disorders suggests that the suppressive capacity of Tregs depends both on the genetic makeup and CSF of patients with psychosis, including drug-naïve patients, are unclear. Il-6 is a pleiotropic cytokine that can signal via both membrane-bound (classic) and soluble (trans) receptors. The classic-signalling is usually associated with its regenerative and anti-inflammatory properties, whereas trans-signalling is frequently associated with its pro-inflammatory actions (Calabrese and Rose-John, 2014). Mendelian randomisation, which uses genetic variants that regulate the levels/activity of a biomarker, suggests a causal role of genetic variants related to Il-6 trans-signalling in psychosis (Hartwig et al., 2017). This indicates that associations are unlikely to be a consequence of confounders, such as lifestyle factors.
function of pro-inflammatory myeloid and effector T-cells. During this inflammatory scenario, IL-6 has a well-studied role, particularly via the trans-signalling pathway, in interfering with de novo generation of functional Tregs by destabilising FoxP3 expression from naïve T-cells (Dominitzki, 2007) and skewing the T-cell fate towards pro-inflammatory Th17 RORc⁺ (Bettelli et al., 2006; Dhuban et al., 2019; Gao et al., 2012; Komatsu et al., 2014).

Interestingly, pharmacological drugs that inhibit IL-6 can restore Tregs in blood. For example, in rheumatoid arthritis (RA) patients, tocilizumab (an anti-IL-6R antibody that inhibits IL-6 pro- and anti-inflammatory pathways) significantly increased blood Treg proportions that correlated with a better response to treatment and clinical state (Kikuchi et al., 2015; Li et al., 2016; Samson et al., 2012). Sarantopoulos et al. (2016) showed that tocilizumab increased blood Tregs in patients soon after the first infusion, an effect sustained over at least a year. In another clinical study, tocilizumab restored the balance between transcriptional factors of Tregs (FoxP3) and Th17 (RORc⁺) (Tada et al., 2016). Thiolat et al. (2014) conducted a mechanistic study in RA patients and rodent models to study the effect of anti-IL-6R antibody on Tregs. They found that mice treated with an anti-IL-6R antibody (MR16-1) showed higher frequencies of Tregs that expressed on their cell surface the ATP-converting enzyme CD39 in both their lymph nodes and spleen. In patients, tocilizumab expanded the percentage of functional CD39⁺ Tregs in the blood of responders versus non-responders as early as a month of treatment. In an experimental in vitro assay, blood CD39⁺ Tregs from responder patients were functionally capable of suppressing the proliferation of pro-inflammatory T cells. Both in the animal and human studies, the results were not a consequence of changes in Th17 frequencies, suggesting that the mechanistic effect of IL-6 inhibition was primarily on the Treg machinery. Ongoing studies are investigating the inhibition of IL-6 pathways in psychosis.

IL-6 blockade is not the only approach with indirect Treg-boosting effects. Others tested for such purpose include mTOR inhibitors (rapamycin, metformin), low-dose IL-2, TNFR2 agonists, rituximab, vitamin D, and others (Copsel et al., 2020; Eggenhuizen et al., 2020; Fisher et al., 2019; Göschl et al., 2019; Rosenzwajg et al., 2019; Sharabi et al., 2018). Flow-cytometry, CyTOF and co-culture assays of Tregs during clinical trials could advance knowledge on novel mechanisms in psychosis, which we discuss in topic 4.

3. Reviewing clinical studies of cellular immune regulation in schizophrenia

3.1. Overview

We identified eight Treg studies. The great majority were cross-sectional studies conducted with medicated patients with schizophrenia. Only one follow-up study included drug-naïve first-episode
### Table 1
Study details.

| Reference | Centre | Design | Sample age (range) | Patients Number | Patients’ details | Comparison group Number and details | Medication | Treg markers | Other immune markers | Cytokines/other markers |
|-----------|--------|--------|--------------------|-----------------|------------------|-------------------------------------|------------|--------------|----------------------|------------------------|
| Maxeiner et al. (2009) | Ulm Germany | Cross-sectional | 14-85 | 17 | Pooled sample of psychiatric patients 28 (not healthy controls) | Patients with neurological disorders 7 Meningitis 5 Chronic Inflammatory 16 Non-Inflammatory 12 Schizophrenia spectrum (F20-25) (duration of illness range: 1-14 years) 5 Affective spectrum (F30-33) (duration of illness range: 1-23 years) | Not mentioned | Membrane CD4, CD25, CD127, CD69 | Membrane CD4, CD8, CD25, CD45RO | Albumin, IgG, IgA, IgM |
| Drexhage et al. (2011) | Rotterdam Netherlands | Cross-sectional Case-control (matched) | 17-39 | 26 | Recent-onset (0-3 years of illness) acutely psychotic in-patients with schizophrenia | All medicated | Membrane CD4, CD25 Intraacellular FoxP3 | Membrane CD3, CD4, CD25, CD45RO Intraacellular IFN-γ, IL-4, IL-17A | IFN-γ, IL-17, IL-10, IL-6, IL-4, TNF-α, CCL2, IL-5, IL-1β, PTX3 (serum cytometric bead array) TGF-β and sIL-2R (sCD25) (ELISA) |
| Fernandez-Egea et al. (2016) | Cambridge UK | Cross-sectional Case-control (matched) | 18-50 | 18 | 18 healthy controls Age-, sex-, BMI-, smoking-matched, zero illegal drug use | Chronic, treatment-resistant schizophrenia (mean duration of illness: 18 years) | 17 Clozapine (mean dose: 316mg/day) 1 Risperidone (long/short) | Membrane CD3, CD4, CD25, CD127 HLA-DR, CD45RA | Membrane CD4, CD25 Intraacellular FoxP3 |
| Kéri et al. (2017) | Budapest Hungary | Follow-up (8 weeks) Case-control (matched) | Mean (SD) SCZ 26.7 (7.0) Healthy control 26.4 (5.5) | 35 | 30 healthy controls Age-, sex- and education-matched | Drug-naive, first-episode schizophrenia (mean duration of illness with no treatment: 8 months) | At follow up (8-week treatment) 24 Olanzapine (10-25mg/day) 11 Risperidone (2-6mg/day) | Membrane CD4, CD25 Intraacellular FoxP3 | Total leucocyte counts CD14 + monocytes CD3 + CD4 + CD25 + activated T cells | TLR (1, 2, 4, 5, 6) expression in monocytes TLR (2, 5) expression in activated T cells and Tregs |
| Cournotte et al. (2018) | Leiden Netherlands | Experimental, random, cross-over Virtual reality controlled experiment | 18-35 | 52 | Pooled sample of ‘Low psychosis liability’ (80 healthy controls 46 healthy controls 34 unaffected siblings of psychosis patients) | Pooled sample of ‘High psychosis liability’ (80 Recent-onset (up to 5 years) 14 Ultra-high-risk psychosis) | Ultra-high risk 9 antidepressant 2 mood stabilizer 2 benzodiazepine Recent psychosis 24 antipsychotics 5 antidepressant 1 mood stabilizer 1 stimulants 9 benzodiazepine Sibling 1 lamotrigine (very low dose) 9 Clozapine | Membrane CD4, CD25 Intraacellular FoxP3 | Membrane CD4, CD25, CD45RO CD4, T CD8, B NK, B cells, monocytes Membrane CD45, CD3, CD4, CD8, CD19, CD1, CD4, CD56, CD15 T (Th1, Th2, Th17) Membrane CD45RO, CD3, CD25, CD4 Intraacellular IFN-γ, IL-4, IL-17A |
| Kelly et al. (2018) | Maryland USA | Cross-sectional | 18-64 | 26 | 17 healthy controls Schizophrenia/ schizoaffective disorder Clinically stable - using the medications 7 Second generation 10 First generation | Schizophrenia/ schizoaffective disorder Clinically stable - using the medications | Membrane CD4, CD25, CD45RO | White blood cell count (total neutrophils, lymphocytes, monocytes eosinophils, basophils) | NA |

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schizophrenia patients. Two studies tested Tregs in vitro. Five studies investigated associations between blood Tregs and symptom domains. We discuss the findings in the following sections. The study details are summarised in Tables 1–3 and Glossary.

### 3.2. Increased Tregs

Overall, half of the studies found higher blood Treg percentages in medicated patients with schizophrenia relative to healthy controls (Drexhage et al., 2011; Kelly et al., 2018; Kéri et al., 2017; Sahbaz et al., 2020). From these, three (Drexhage et al., 2011; Kéri et al., 2017; Sahbaz et al., 2020) observed that Treg percentages (CD4+CD25+FoxP3+) in patients were increased concomitantly with greater percentages of activated (CD3+CD25+) or pro-inflammatory (CD4+IL17A+CD45RO+) T-cells. In contrast, Kelly (2018) found increased Treg percentages in clinically stable patients with schizophrenia compared with controls in the absence of elevated proportions of CD3+ or CD4+T-cells, but pro-inflammatory subsets were not evaluated.

Drexhage et al. (2011) performed additional analyses by stratifying their sample based on an inflammatory gene expression signature in monocytes; they found that 11/19 patients and 3/23 controls were in an inflamed monocyte state. They further observed that the dysregulated adaptive immune phenotype of upregulated Tregs alongside higher pro-inflammatory Th17 percentages in patients occurred irrespective of the inflamed innate monocyte state (although slightly more evident in the negative monocyte subgroup), which they interpret as reflecting an altered T-cell set point in schizophrenia.

### 3.3. Decreased Tregs

Three studies (Drexhage et al., 2011; Fernandez-Egea et al., 2016; Maxeiner et al., 2009) identified lower blood Tregs in subgroups of medicated patients versus controls. In the sample from Drexhage et al. (2011), although blood Treg percentages were overall increased in patients than controls, around one-third of the patients had low Tregs (<3.25% cut-off). Fernandez-Egea et al. (2016) performed detailed flow cytometry of 73 immune cells in treatment-resistant schizophrenia patients medicated with clozapine versus controls. Using partial least squares to predict diagnosis, they identified two distinct immune phenotypes. The first comprised lower numbers of activated and memory Tregs (evaluated by surface markers HLA-DR+ and CD45RA+, respectively) in patients versus controls. The second consisted of higher numbers of classical monocytes (CD14+CD16+) and CD8+CD45RA+ among neurological patients, accompanied by greater disinhibition of the systemic T-CD4+/CD8− ratio. The blood and liquor Treg phenotype overlapped with a cluster of patients with inflammatory neurological conditions, suggesting a potential shared inflammatory process driven by the disturbed Treg populations. However, the pooled psychiatry sample and the lack of a control group hamper further interpretation.

### 3.4. Unchanged Tregs

One study reported no difference in blood Tregs (CD3−CD4−FoxP3+) or Th17 frequencies in recent-onset treated
Table 2
Immune cells results.

| Reference | Tregs | Other immune cells | Subgroups | Cytokines/Other markers | Confounding | Notes |
|-----------|-------|-------------------|-----------|------------------------|-------------|-------|
| Maxeiner et al. (2009) | Psychiatric patients vs Neurological patients | Psychiatric patients vs Neurological patients | Tregs | 10/17 Psychiatric patients with Treg percentages (CD4+CD127dim) higher than 10% in the CSF and lower than 10% in the blood vs non-inflammatory neurological patients; this subgroup of psychiatric patients overlapped with a cluster of inflamed neurological patients | Albinum, IgG, IgA, IgM | All lower vs. meningitis patients | Not adjusted |
| Drexhage et al. (2011) | SCZ vs. HC | SCZ vs. HC | Tregs | One-third of patients (n=77) with lower blood Tregs (<3.25%) | SCZ vs. HC | High sII-R | Adjusted for sex and age (matched) |
| Fernandez-Egea et al. (2016) | SCZ vs. HC | SCZ vs. HC | Other immune cells | Lower functional, activated (HLA-DR+) memory Tregs (CD3RA-) | Immune cluster 1: Lower functional, activated (HLA-DR+) memory Tregs (CD3RA-) | NA | Adjusted for age, sex, BMI, and cigarette smoking (matched) |
| Kelly et al. (2018) | SCZ vs HC | SCZ vs HC | Tregs | Monocytes (CD16+CD16+CD45RA+) and memory T-cells (CD4+CD45RA+) | MFI (TLR density on cell membrane) | Age, sex, and schooling (matched), medication (patients) | Results expressed as the percentages of cells expressing each TLRs, and MFI for TLR density on cell membrane |

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Table 2 (continued)

| Reference | Reference | Results | Subgroups | Cytokines/Other markers |
|-----------|-----------|---------|-----------|-------------------------|
| Cournotte et al. (2018) | High vs low-psychosis liability | NA | NA | Adjusted for age, sex, ethnicity, education level, smoking, psychotropic medication |
| | NS | NA | T cell subsets expressed as percentages of total lymphocytes were counted and stored in liquid nitrogen |
| | CD4+CD24+FoxP3+ | NA | SCZ vs. HC | Adjusted for age, sex, smoking, BMI medication (patients) |
| | CD25+FoxP3+ | NA | SCZ vs. HC | Results represent the percentages, not numbers, of cells within the lymphocyte population |
| | CD25+CD45RA+ (memory Tregs) | NA | SCZ vs. HC | |
| Kelly et al. (2018) | SCZ vs. HC | Lower astrocyte CCL20 gene expression = astrocytes less capable of inducing CCR6+ Tregs chemotaxis | NA | |
| | High | White blood cell counts, CD3+ or CD4+ | SCZ vs. HC | |
| | NS | | NS | |
| Akkouh et al. (2020) | iPSC-astrocyte SCZ vs. controls (after in vitro IL1β challenge) | Low FoxP3 mRNA in whole blood | NA | |
| | Lower astrocyte CCL20 gene expression = astrocytes less capable of inducing CCR6+ Tregs chemotaxis | NS | CCR6 mRNA in whole blood | |
| | SCZ vs. HC | NA | | |
| | High | Plasma | | |
| | NS | Adjusted for age, sex, ethnicity, BMI education level, smoking status | Results represent the percentages, not numbers, of cells within the lymphocyte population |
| | SCZ vs. HC | Adjusted for age, sex, ethnicity, BMI, education, smoking status | |
| | SCZ vs. HC | Adjusted for age, sex, smoking, BMI medication | |
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BMI: body mass index; CSF: cerebrospinal fluid; DUP: duration of untreated psychosis; HC: healthy control; TLR: Toll-like receptor; NA: non-applicable; NS: non-significant; PBMCs: peripheral blood mononuclear cells; SCZ: schizophrenia.

Results reflect differences in the peripheral blood, unless otherwise indicated.

Significant results are highlighted in bold.

3.5. A longitudinal study

A single follow-up study included drug-naïve first-episode schizophrenia patients and healthy controls, in which the authors measured the...
Table 3
Association between Tregs or other immune cells and clinical and stress variables in patients.

| Reference | Clinical instruments | Immune cells and clinical/ stress variables correlations in patients |
|-----------|----------------------|---------------------------------------------------------------|
| Maxeiner et al. (2009) | SCID-1 (DSM-IV) | Not evaluated |
| Drexhage et al. (2011) | DSM-IV criteria (CASH interview) | GAF at discharge |
| Fernandez-Egea et al., 2017 | Structured interview ICD-10 standards | Positive correlation between %Tregs at admission and GAF at discharge in subgroups Treg > 3.25% (n=16) better GAF ≥ 50 at discharge |
| Kéri et al., 2017 | Structured Clinical Interview for DSM-IV (SCID-CV) | Immune cluster 1 including lower Tregs (HLA-DR+ CD45RA-) accounted for 53% of diagnostic variance |
| (Cournette et al., 2016) | CAARMS | Clinical variables |
| | GPTS | Not evaluated |
| | SIAS | Significant psychosis liability x CTQ for Th17 only |
| | CAPE | In high-psychosis liability subgroup, childhood trauma associated with increased %Th17 |
| | CTQ-SF | Virtual stress |
| | SCID-V | Significant psychosis liability x subjective stress to social virtual experiment for Tregs and NK cells |
| Kelly et al. (2018) | SANS - negative symptoms | SANS - negative symptoms |
| | BPRS - global | High %Tregs correlated with lower negative symptoms (total SANS), driven primarily by alogia and affective blunting |
| | psychopathology and positive symptoms | |
| | CD- depressive symptoms | |

Table 3 (continued)

| Reference | Clinical instruments | Immune cells and clinical/ stress variables correlations in patients |
|-----------|----------------------|----------------------------------------------------------------|
| Akkouh et al. (2020) | SCID-1 (DSM-IV) | PANSS |
| Sahbaz et al. (2020) | SCID - DSM-IV | %Treg NS |
| | | Supernatant IL-4 and IL-10 negatively correlated with PANSS negative scores |
| | | %CD19+ and CD20+ B cells positively correlated with PANSS total and global scores |

BACS: The Brief Assessment of Cognition for Schizophrenia; CASH: The Comprehensive Assessment of Symptoms and History interview; CGI-S: Clinical Global Impression for Schizophrenia; CAARM: Comprehensive Assessment of At-Risk Mental States; CAPE: Community Assessment of Psychotic Experiences; CDS: The Calgary Depression Rating Scale; CTQ-SF: Childhood Trauma Questionnaire Short Form; DSM: Diagnostic and Statistical Manual of Mental Disorders; GAF: Global Assessment of Functioning; GPTS: Green Paranoid Thoughts Scale; ICD: International Classification of Diseases; MATRICS-MCBB: The MATRICS Consensus Cognitive Battery; OPCRIT: Operational Criteria Checklist for Psychotic Illness and Affective Illness; PANSS: Positive and Negative Syndrome Scale; RBANS: Repeatable Battery for the Assessment of Neuropsychological Status; SANS: The Scale for the Assessment of Negative Symptoms; SIAS: Social Interaction Anxiety Scale. Significant results are highlighted in bold.

3.6. Functional capacity of Tregs in vitro

Akkouh et al. (2020) found evidence for dysregulated astroglia-Treg axis in schizophrenia. Astrocytes cultures from patients' fibroblasts secreted less CCL20 – a chemokine that attracts Tregs to the CSF. In addition, they reported, in a large independent sample (n > 800), that patients had lower expression of the Treg-specific transcriptional factor FoxP3 in their whole blood than controls. However, the connection between the two findings is obscure, and the assessment of Tregs in patients' blood in this study was only indirect through the measurement of FoxP3 mRNA.

Sahbaz et al. (2020) tested for the first time Tregs competence to regulate pro-immune responses in chronic patients and controls. Although Treg percentages were increased in patients versus controls in unstimulated conditions, after in vitro stimulation with both anti-CD3 and anti-CD28 (a cocktail that triggers intracellular signalling and stimulates T-cell proliferation), the patients regulatory arm had a weaker proliferative response than the controls (also in within-subject analyses) accompanied by increased pro-inflammatory cytokines in culture supernatants (IL-6, IL-17A, IFN-γ, TNF-α). This is the only investigation testing the proliferative capacity of Tregs in psychosis, requiring replication in future, ideally, prospective studies.
3.7. Circulating Tregs and correlations with psychosis symptom domains

The results are summarised in Table 3. There is evidence that low blood Tregs are associated with worst symptomatology. Drexhage et al. (2011) found low blood Treg percentages in one-third of their patient sample at admission that was associated with less favourable global functioning at discharge. Fernandez-Egea et al. (2016) found that more severe negative and cognitive symptoms in treatment-resistant schizophrenia were associated with reduced numbers of experienced Tregs. Finally, Kelly et al., 2018 found that remitted patients with schizophrenia had high blood Treg percentages that were significantly associated with fewer negative symptoms (alogia and affective blunting) and a trend to better cognitive performance (reasoning, problem-solving, processing speed). While in Sahbaz et al. (2020) Treg percentages were not associated with symptomatology, the anti-inflammatory IL-10 (detected in cellular supernatants of Treg assay) was negatively correlated with negative symptoms.

4. Conclusions and future perspectives

4.1. Implications for the h-Treg hypothesis

We discussed the obvious general shortcomings of the literature; studies are few with small samples conducted in medicated patients, and poorly adjusted for the effects of confounding such as body mass index (BMI) and tobacco smoking, and no clinical follow-up to test possible causal mechanisms. Nevertheless, we suggest that the h-Treg hypothesis is compatible with and encouraged by two key aspects of the literature. First, four studies report that greater presence of Tregs is associated with better clinical status in treated patients. This allows that h-Tregs may have a state-dependent causal role in psychosis that could be tested in longitudinal studies in minimally treated patients while adjusting for confounding (e.g., sex, age, BMI, tobacco, and other drugs). A further implication is that other studies reporting increased Tregs in patients without clinical measures do not refute the h-Treg hypothesis. Second, Sahbaz et al. (2020) provide a strong test of the hypothesis by directly measuring the ability of Tregs to limit inflammatory responses to an in vitro challenge and finding it impaired.

We make specific suggestions for further tests of the h-Treg hypothesis below. However, we would reiterate a concluding point from our original proposal that Treg dysfunction will not be the sole primary abnormality in the causation of psychosis. It is clear that a multi-level complex of an interacting multiplicity of factors will be involved, perhaps converging on some common pathways such as Treg functionality, which may vary across functional subtypes of psychosis and transdiagnostic disorders.

4.2. Better measures of Treg function

The heterogeneity of markers used to detect Tregs in blood across studies may obscure consistent group differences. Tregs are usually identified as CD4+CD25+FoxP3+; however, effector CD4+ T-cells can also express CD25. Moreover, FoxP3-expressing Tregs are mixed populations, with some having a minimal suppressive activity. Such limitations have prompted the search for additional markers, such as CD127. Combined CD4+CD25+FoxP3+CD127low has improved Treg purification and should be used for consistency (Liu et al., 2006).

While there is no such ‘best’ functional marker, different molecules combined to CD4+CD25+FoxP3+CD127low can help to delineate Tregs with highly suppressive activity (Hou et al., 2019). For example, Fernandez-Egea et al. (2016) identified Treg subpopulations with more suppressive function (HLA-DR+CD45RA-) that were reduced in treatment-resistant patients. Other functional markers include CTLA4, GARP, TNFR2, and others (Table 4). Additionally, CD39 and CD73 are attractive in autoimmune disorders for their key role in adenosine synthesis, a potent anti-inflammatory molecule (Peres et al., 2018b).

Techniques such as CyTOF permit concurrent detection of more than 40 different antibodies with high-dimensional single-cell proteomic data (Subrahmanyam and Maecker, 2021) that could be useful in future Treg studies in psychosis.

One important consideration is that counting the proportion of blood Tregs is not a direct measure of their functional capacity. The study from Sahbaz et al. (2020) is a great example and, so far, the only testing the proliferative capacity of Tregs in vitro. As in autoimmune disorders (Cribbs et al., 2015; Thiolat et al., 2014), assays of Tregs and T-effector cells (alone and co-culture), determination of cytokines in supernatant, and DNA methylation of the FoxP3 locus are attractive approaches, and perhaps more precise, to test the functional capacity of Tregs. This could be applied to psychosis, ideally in longitudinal studies of untreated patients with placebo control to determine the hypofunctional state of Tregs and ascertain causality.

4.3. Clinical correlates of Treg function in trials of early psychosis

Large clinical trials are needed to move beyond correlational studies and identify the causal roles of immune-regulatory dysfunction in the pathogenesis of psychosis. Any trial of new or repurposed agents, especially those targeting immune mechanisms, should include cellular immunophenotyping together with traditional cytokine measures to develop immune markers of illness subtypes, severity, and stratification.

Table 4

| Functional markers | Ligands | Main function/contributions |
|--------------------|---------|-----------------------------|
| CTLA4 (CD152)      | CD80/CD86 (B7) | Negatively regulates immune responses; restrains CD28 engagement by competing with B7 on antigen-presenting cells |
| HLA-DR+           | – | High FoxP3-expressing Tregs (highly suppressive) |
| GARP (or LR8C32)  | TGF-β | A transmembrane protein expressed in activated Tregs that promotes TGF-β release and activation |
| TNFR2             | – | Activates and expands Tregs (highly suppressive) |
| GITR – glucocorticoid-induced receptor family protein | TGF-β | Promotes TGF-β activation and mediates Treg-immune suppression |
| Integrin αvβ8     | CD39 | ATP and ADP Converts ATP and ADP to AMP |
| CD73              | CD155/CD112/PDL1/PDL2 | Converts AMP to adenosine innate and adaptive cells |
| TIGIT             | – | Activates and expands Tregs (highly suppressive) |
| CD123             | CD112 | Negatively regulates immune responses; inhibits CD28 and TCR signalling |
| CD45R+CD45R0+     | CD40/CD86 (B7) | Co-stimulatory molecule; promotes T cell activation, proliferation, and survival |
| CD45RA-CD45RO+    | – | Memory markers |
| CD45RA+CD25+FoxP3+ | – | Naïve-resting Tregs; predominantly thymus-derived (moderate suppressive activity) |
| CD45RA-CD25+FoxP3high | – | Activated-memory Tregs; predominantly thymus-derived (highly suppressive) |
| CD4+CD25+HLA-DR+Tregs | – | Tregs with activated and highly suppressive phenotype |
| CD45RA-FoxP3low | – | Non-Tregs (Th17 signature with BORc upregulation) |
Imaging methods, such as DTI, magnetic resonance imaging (MRI), and functional MRI could be used to assess the relationship between structural and functional brain changes with blood Tregs and other leukocyte populations, including the Treg:Th17 ratio (Poletti et al., 2017), along with careful standardised clinical assessments of symptoms and social and occupational function using transdiagnostic methods. Besides, new PET radioligands (Beano et al., 2017) are emerging that can be used to assess the functional status of astroglia, microglia, and neuron function in relation to baseline and changes in Tregs function. There is also need for better adjustment for confounding as only half of the studies adjusted for the effects of BMI and smoking in addition to sex and age (Table 2). The identification of genetic variants associated with Tregs activity/function could be useful in future mendelian randomisation studies to understand potential causality and address issues of confounding and reverse causation.

4.4. Hypofunctional Treg hypothesis of psychosis: a role for IL-6?

Associations between low Tregs and worst symptomatology could be reflecting a hyperactive IL-6 state observed in subgroups of patients. We summarise some key mechanisms in Fig. 3. To disentangle that, clinical trials testing the efficacy of IL-6 blockers in psychosis could assess Tregs immune phenotype in vivo and test their suppressive capacity with in vitro co-culture assays before and after IL-6 blockade. Such analyses combined with the Treg:Th17 ratio can help to elucidate mechanisms of IL-6-induced Treg destabilisation. Given the pro-and anti-inflammatory roles of IL-6, in vitro assays are feasible to assess Tregs suppressive capacity in response to drugs that inhibit both- (e.g., tocilizumab) or only the pro-inflammatory pathway of IL-6 (e.g., olamkicept).

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Authors contributions

F.C.-Z. and B.D developed the hypofunctional Treg hypothesis. F.C.-Z. wrote the manuscript, designed the tables and figures. B.D. supervised the work and together with the other authors critically revised the manuscript. The figures were designed using a premium account from Biorender (https://biorender.com/).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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