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Review

The Role of T-Regulatory Cells in the Immune System and Their Therapeutic Potential in Inflammation and Lung Cancer

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Abstract: One of the key links of the immune system, which plays a crucial role in maintaining homeostasis and lung function, is T-regulatory cells (Treg). Their suppressive effect on the immune system predetermines the potential of various modulating effects, taking into account the pathogenetic features of the pathology, which underlie innovative methods of therapy, including in pulmonology. However, awareness of them remains insufficient and, in some cases, contradictory, which requires appropriate consideration and analysis, which became the purpose of this work. An analysis of 69 literary sources selected from different databases of biomedical scientific information was carried out. The analysis showed that targeted methods for the treatment of inflammation and tumors are developed based on the generally accepted paradigm about Treg and are aimed at their modulation. In inflammation, activation of Treg, exerting a suppressive effect on T-effector cells, reduces it. In tumors, their depletion, promoting the expression of T-effector cells, including antitumor cells, slows their growth. This is achieved by various direct or indirect methods of influence. Along with this, some researchers suggest using a depleting effect on Tregs in inflammation, and similarly, in both tumor and inflammatory processes, which contradicts the generally accepted paradigm regarding Tregs. Thus, a modulating effect on Tregs can have a therapeutic effect on both inflammation and tumors, with a differentiated consideration of the mechanisms of their development. Methods of treating inflammation that contradict the generally accepted paradigm regarding Tregs remain unsupported by a sufficient evidence base and are untenable.

Keywords: T-Regulatory Cells; Adoptive Transfer; IL-2, Inflammation; COVID-19; Autoimmune; Oncological Processes; Cytostatics

1. Introduction

The central link of the immune system, T-regulatory cells (Treg), plays a crucial role in regulating its response to pathogenic factors, which is important for understanding the development and therapeutic control of inflammatory processes and tumors, significant issues in pulmonology [1]. A paradigm has emerged regarding the role of Tregs in maintaining immune system homeostasis, highlighting their significant impact and therapeutic potential. However, the potential of using Tregs from the standpoint of their therapeutic applications and their influence on inflammation and tumors in the lungs is not sufficiently reflected in the literature, which limits their practical use in pulmonology [2]. At the same time, individual researchers (IR) rely on different ideas and propose approaches to the therapeutic effect on inflammation in the lungs that contradict this paradigm. All this obliges us to consider the problem of Treg and approaches to correcting the immune system and its methods from the perspective of

the T-regulatory link and the optimization of treatment for inflammatory and tumor processes in the lungs, which determines the purpose of this work.

2. Materials and Methods

The selection and subsequent analysis of publications posted on PubMed, Embase, Cochrane, and Index Medicus platforms, as well as in the clinicaltrials.gov registry of clinical trials, were carried out using keywords and phrases, including immunosuppressants, inhalation administration, COVID-19, hyperinflammation, and Treg. The following keywords or phrases were used for the selection: Treg, adoptive transfer, IL-2, inflammation, COVID-19, autoimmune and oncological processes, cytostatics. The analysis included 69 full-text sources of literature, with narrowing at each stage. The selected data were then structured and used (**Figure 1**).

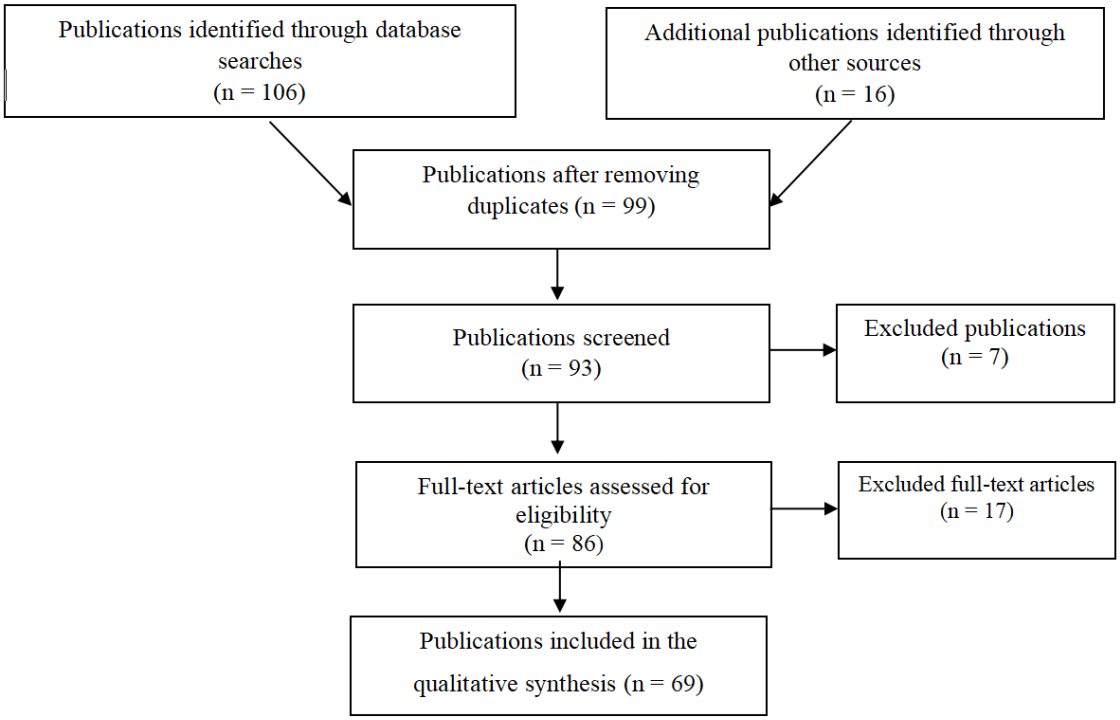

Figure 1. Scheme of publication selection.

3. The Role of Tregs in Immune System Homeostasis in Inflammation and Lung Cancer

Tregs are a specialized subset of T cells that play a major role in preventing/limiting excessive immune responses and, by creating a balance with T effector cells (cytotoxic T lymphocytes, CD8+ T cells, and natural killers, otherwise NK cells, T helpers, macrophages), ensure homeostasis of the immune system. Cytotoxic T lymphocytes recognize and bind to cells that carry foreign antigens on their surface, releasing cytotoxic substances (perforins and granzymes) that cause apoptosis in the target cell. They play a key role in the fight against viral infections and tumors. T-helper cells play an important role in the activation and regulation of cytotoxic T-lymphocyte and NK cell activity [3]. Macrophages are involved in the destruction of captured pathogens and the activation of other immune cells [4]. Tregs contribute to the resolution of acute inflammation and prevent the development of chronic inflammation, which plays a significant role in carcinogenesis [5]. Suppression of immunity is necessary to prevent autoimmune processes and ensure that the immune system does not attack the body's tissues [6]. Based on experimental data and clinical studies, it has been established that Tregs play a central role in suppressing the immune response and promoting resolution in lung damage caused by acute pneumonia, sepsis, or inhalation injury, the most pronounced consequence of which can be acute respiratory distress syndrome (ARDS). At the same time, it has been shown that a decrease/absence of Tregs leads to more severe lung damage, delayed recovery, and high

mortality [7]. But along with suppressing immunity during inflammation, they can also suppress antitumor immune responses, which contributes to tumor progression. It has been established that Tregs are present in large quantities in the microenvironment of lung cancer and, by suppressing cytotoxic T lymphocytes and NK cells, which are crucial for fighting cancer, significantly suppress antitumor immunity, which is aggravated by the suppression of antigen-presenting cells, allowing cancer cells to bypass immune surveillance, grow and spread [8, 9]. It is on these features of Tregs that the strategy of therapeutic use of action on them is based.

4. Possibilities and Prospects for Correcting Inflammation by Acting on Tregs in Different Ways

Based on the generally accepted paradigm regarding the role of Tregs in regulating the immune system, various actions have been taken to increase Tregs in different ways for therapeutic purposes to treat inflammation. One of them is direct autologous or allogeneic (donor, cord blood) adoptive transfer of Tregs [10,11]. Another indirect method may be the use of low doses of IL-2, which activates Tregs, especially in autoimmune processes where the immune system attacks healthy tissues [12,13]. At the same time, even though IL-2 is a proinflammatory cytokine and can activate other immune cells, which can cause undesirable side effects, the effect of its action can be positive, which once again indicates that in this case, an increase in Tregs for the regulation of hyperinflammation and its relief under the influence of IL-2 is much more significant than a possible proinflammatory effect. Causing both an immunogenic and tolerogenic immune response, it actually has a pleiotropic effect characteristic of cytokines [14,15]. At the same time, on the one hand, it ensures the "life" of Tregs and their quality, and on the other hand, it promotes their influx into the area of inflammation due to its proinflammatory properties. That is, its effect in this case is an increase in the number and functional activity of Tregs. And if we consider the possibility of predominantly using one of these actions, then its use in the form of a slight increase can become therapeutically quite effective without unwanted side effects; more on this below in the relevant section. These technologies are gaining significant success and recognition, which indicates, on the one hand, the adequacy of this idea of the mechanisms of regulation of the immune response, in which Tregs play a leading controlling role, and on the other hand, good therapeutic possibilities of influencing the inflammatory process using methods aimed at increasing them. Let us delve into the possible therapeutic effects of these technologies in more detail.

4.1. Increasing T-regulatory Cells for the Treatment of Pneumonia

Based on **Figure 2**, which summarizes, according to the analysis, the role of Tregs in the development of hyperinflammation and the immune system's response to it, the following can be noted.

Perhaps, at the early stage of the COVID-19 infectious process, an increased number of activated Tregs can reduce antiviral protection, exerting an immunosuppressive effect on protective immune cells and suppressing the immune response against SARS-CoV-2 (**Figure 2A**). Consequently, the administration of suppressive drugs, particularly cytostatics, may be pointless. However, the vast majority of studies indicating Treg activation in COVID-19 patients are evidence that this is, in fact, a protective mechanism that attenuates inflammatory responses and suppresses the cytokine storm, leading to a less severe form of the disease and recovery even if it is protracted (**Figure 2B**). A decrease in Treg in the late phase of COVID-19, characterized by hyperinflammation, or perhaps under the influence of some targeted impact, is characterized by the opposite effect. In this case, the activation of proinflammatory immune cells and an increase in the production of proinflammatory cytokines occur, which cause or exacerbate the cytokine storm and damage to the body's tissues, leading to acute respiratory distress syndrome and often death (**Figures 2C** and **3A**). It has been established that the ratio of the two main subpopulations of CD4 T cells - immunosuppressive T cells and proinflammatory Th17 T cells - largely determines the course of the inflammatory process. A decrease in T cells, causing a shift in the balance of these cells towards Th17 T cells, stimulates the synthesis of other proinflammatory cytokines that promote hyperinflammatory reactions [16,17]. Conversely, stimulation and increase of Tregs, with a shift in the balance towards Tregs, reduce inflammatory responses and the incidence of mortality (**Figures 2B** and **3C**). The concepts outlined above are consistent with the results of targeted stimulatory effects with different mechanisms on Treg potential (**Figure 3**) and a shift in the Th17/Treg balance towards Tregs, which may be utilized for effective therapy of the inflammatory process in COVID-19 and will be discussed below. Possible options for influencing Tregs and the inflammatory and tumor processes are shown in **Figure 3**.

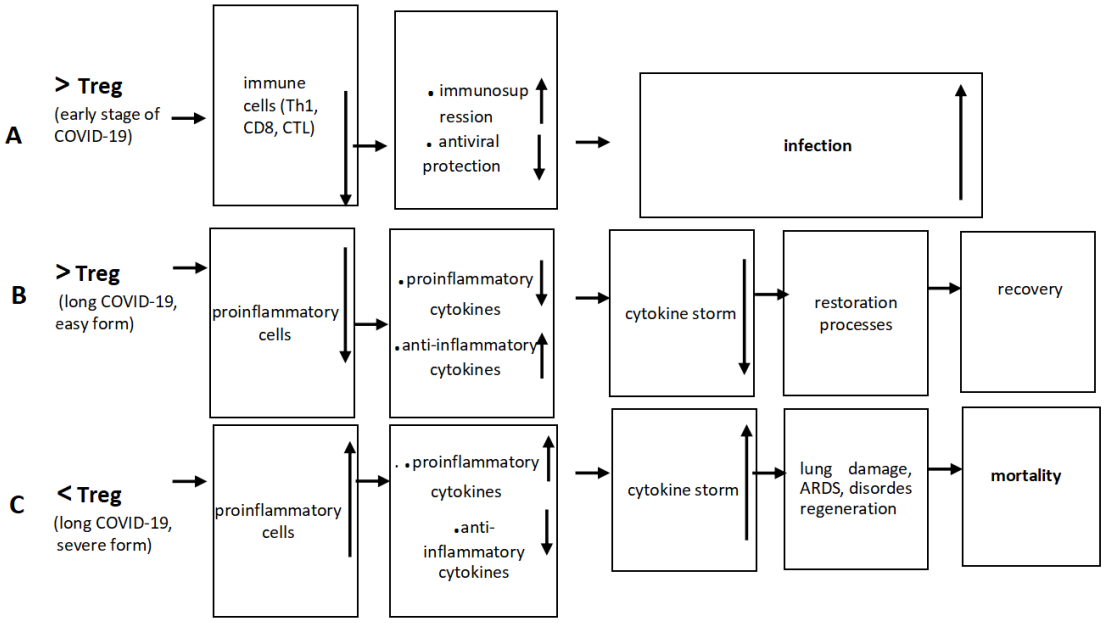

Figure 2. The role of Treg in the pathogenesis of COVID-19.

Notes: > /, up arrow – activation/increase; < /, down arrow – suppression/reduction.

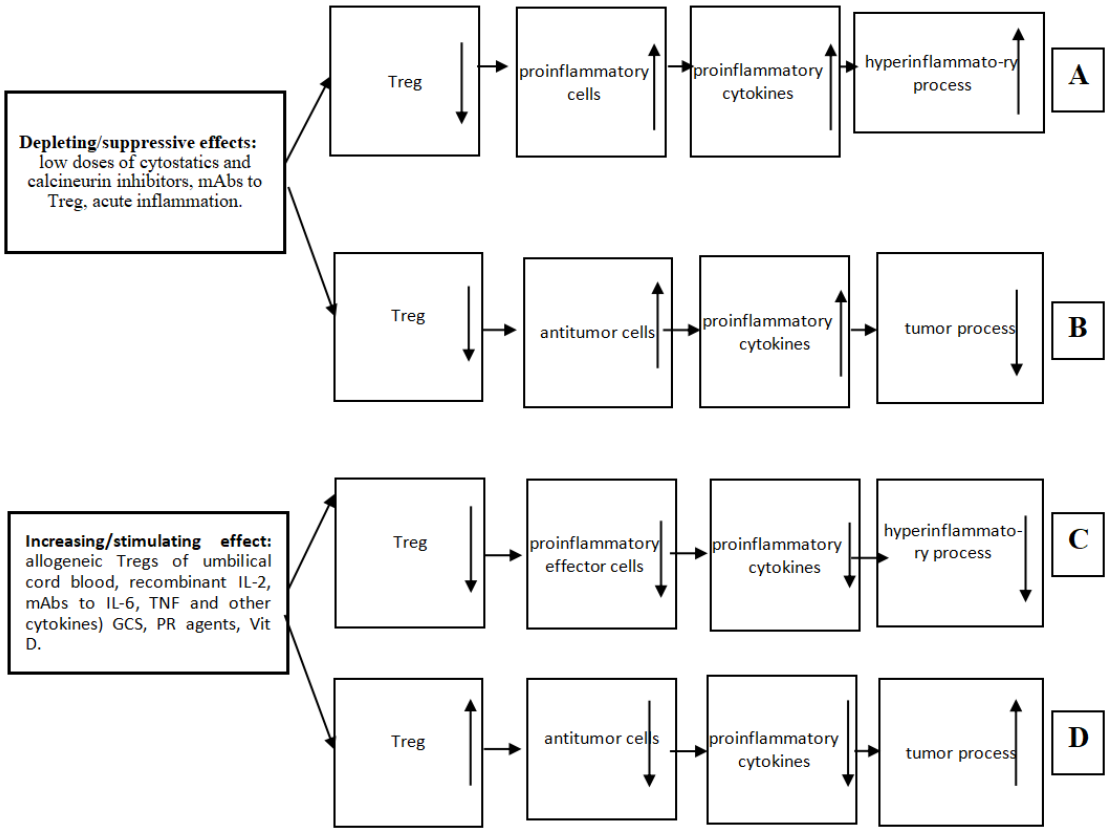

Figure 3. Therapeutic potential of modulating effects on Tregs in inflammation and tumors in the lungs.

Notes: > /, up arrow – activation/increase; < /, down arrow – suppression/decrease; A, B – in inflammation; C, D – in oncology.

And if a decrease in Treg contributes to an adverse effect from the immune system, manifested by an exacerba-

tion of inflammation, then in the oncological process, on the contrary, it can have a positive effect, since antitumor cells are initiated, which helps to stop the tumor process and its more favorable course [18]. That is, the action of Treg is multidirectional, and to obtain a therapeutic effect in these processes, it is necessary to increase Treg in the case of inflammation and deplete Treg cells in the oncological process. This understanding underlies the therapeutic effect through external regulation of Treg, in contrast to the interpretations of IR, which are somewhat different from the accepted paradigm for Treg or are directly opposite to it [19–23].

4.2. Targeting IL-2 in the Treatment of Lung Inflammation

In recent decades, there has been renewed interest in IL-2 immunotherapy driven by structural and biophysical understanding of the complex formed by IL-2 with its IL-2 receptor (IL-2R) subunits and the discovery that IL-2 at low doses or through modification can be used to selectively stimulate either cytotoxic effector T cells or Treg cells [24].

Clinical trials using low doses of IL-2 have demonstrated the potential of IL-2 to expand Tregs and modulate Treg-deficient immune pathologies [25–27]. Low-dose IL-2 therapy has shown promise as an immunotherapy for the treatment of a wide range of autoimmune and inflammatory diseases. For a clearer understanding of the mechanisms of using the effect on Tregs with IL-2, it is appropriate to note that there is a certain analogy with the use of ultra-low doses of cytostatics proposed by the IR, which explain their action by the exclusion of toxic effects and side effects while reducing the proinflammatory effect of IL-2 on T-effector cells (Tef) [12–16]. However, this overlooks the main point that the suppression of IL-2R contributes to a decrease in the main link of the immune system, Tregs, which exacerbates inflammation and leads to the development of autoimmune reactions through the initiation of Tef. On the contrary, the use of low doses of IL-2 contributes to an increase in Tregs, given their selective high sensitivity to the level of IL-2. Therefore, without increasing Tef activity, it leads to a decrease in inflammation (**Figure 4**). IL-2 modification has led to the creation of IL-2 preparations with improved and selective immunostimulatory capabilities [25]. IL-2-mediated modulation of immune responses using IL-2/monoclonal antibody (mAb) complexes highlights the potential of using IL-2 muteins to antagonize rather than stimulate IL-2R-induced signaling [28].

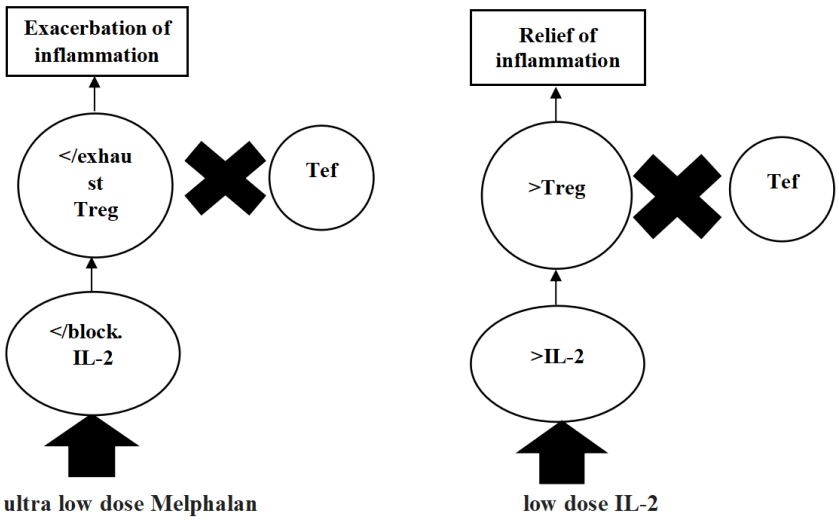

Figure 4. Comparative assessment of the impact of ultra-low doses of cytostatics and low doses of IL-2 on the T-regulatory link of the immune system according to the existing generally accepted paradigm.

Although recent advances in structural modification or modulation of IL-2 have revolutionized the use of IL-2 in immunomodulatory processes that require strict Teff selection, such as stimulating Tregs for tolerance or activating CD8+ T cells against infection and cancer, low-dose IL-2 therapy is one such outcome and has proven to be a promising immunotherapy for the treatment of a wide range of autoimmune and inflammatory diseases. Thus, for the inflammatory process, Tregs are an important protective mechanism that ensures the limitation of inflammation

and the initiation of repair processes, especially in conditions of hyperergic reaction, when their function is compensatorily enhanced by increasing their activity and number, leveling out inflammation and lung injury. Their reduction, on the contrary, contributes to an increase in Teff activity and the development or aggravation of an already existing increased immunological response, negatively affecting inflammation, but so necessary for the activation of antitumor cells, contributing to the suppression of the tumor, which has a different pathogenetic direction relative to inflammation, which is not taken into account by IR [19–23]. The very fact of a significant positive effect on the inflammatory process of technologies for targeted increase in Treg and decrease in Teff activation in different ways and on the oncological process of methods for targeted decrease in Treg and increase in Teff activation, on the one hand, is once again convincing confirmation of the validity of the generally accepted paradigm regarding Treg, and on the other hand, indicates the importance and effectiveness of using various methods that enhance Treg to reduce the level of hyperinflammation and tissue damage, especially in severely infected patients with COVID-19 and elderly people with weakened immunity, in whom the level of Treg is usually reduced or does not function. Along with this, the opinion of the IR and the approach and method proposed by them are opposite to the generally accepted paradigm, which, based on the materials they published, scientific reports and discussions, often the absence of objective research and assessment methods, although declared by them, without sufficient evidence, interpret that, on the contrary, a decrease in Treg cells is necessary to stop the process. Such an interpretation of the role of Treg a priori distorts existing ideas and the generally accepted paradigm. Accordingly, the proposed and assumed impact of cytostatics on the IR neutralizes only the potential capabilities of important immune system links, as their use optimizes the balance in this system and treats inflammation and the tumor process. It should be taken into account that alkylating cytostatics, particularly cyclophosphamide and melphalan, especially in ultra-low doses, significantly disrupt the signal transmission of the IL-2R receptor to IL-2 and inhibit Treg, as IL-2 is not only a growth factor but also, as noted above, a survival factor for them. A similar effect may also occur when using alkylating cytostatics with respect to other surface cell receptors, including TNFR II (tumor necrosis factor receptor type II) and the Fas receptor [29]. A decrease in the number and suppression of the function of Treg cells against the background of a decrease in the expression of anti-inflammatory and antitumor cytokines under the influence of low doses of alkylating agents was also noted by other researchers, in particular, those conducting morphofunctional, immunological, and genetic studies of BAL [30,31]. In this case, a pronounced exacerbation of the inflammatory process was observed [32], which is consistent with the generally accepted paradigm. However, the results of alkylating cytostatics in these IRs, which run counter to the generally accepted paradigm and suggest, according to it, a negative effect in the form of exacerbation (especially when delivered to the lungs in the form of aerosols and low doses), were similar to those obtained using technologies corresponding to the generally accepted paradigm, and even exceeded them, which raises some doubts. High doses of cytostatics lead to immunosuppression, whereas it has been shown that low doses enhance the immune response by suppressing Tregs and reducing control, which leads to an excess of Teff and a significant enhancement of the immune response, and accordingly, to an increase in the activity of antitumor cells. A large number of studies in the context of antitumor vaccines have focused on the inhibitory effect of cytostatics on Tregs. However, such activation of the immune response contributes to the exacerbation of the inflammatory process, rather than its remission. Moreover, low doses of alkylating cytostatics can enhance this effect by increasing both T cell and memory T cell proliferation, as well as the production of inflammatory mediators such as GM-CSF, IL-1β, IL-5, IL-10, IFN-γ, and tumor necrosis factor-α in the tumor microenvironment [33–35].

Thus, it is important to comprehend the opposing effects of Treg modulation, as immunostimulation is desirable in cancer therapy but not in the treatment of inflammation and autoimmune diseases, whereas immunosuppression is suitable in the treatment of inflammation and autoimmune diseases but not in cancer therapy [36].

Depletion or decrease of Tregs can lead to the development of autoimmune processes due to various factors and their combination. It should be emphasized that, on the one hand, COVID-19 is associated with a high risk of developing autoimmune diseases. This has been demonstrated by population cohort studies and is explained by various theories, including molecular mimicry of viral proteins, systemic manifestation and multiorgan damage of COVID-19 due to the widespread expression of the SARS-CoV-2 ACE2 receptor, activation of autoantigen release from virus-damaged tissues, lymphocyte activation, and epitope dissemination [37,38]. On the other hand, as shown above, the presence of COVID-19 is associated with a decrease in Tregs, which is linked to the development of autoimmune diseases. That is, these two factors potentiate the risk of autoimmune diseases. There-

fore, the depletion of Tregs provided by the IR, in addition to the lack of indications for it and a possible negative impact on the inflammatory process, creates an unjustified additional threat of side effects. It has been shown that melphalan, having a lymphodepletion and myelodepletion effect used in CAR (chimeric antibodies receptor)-T therapy, neutralizes the suppressive activity of Tregs, has an immunostimulating effect, promoting the release of proinflammatory cytokines, including IL-1, IL-33, IL-6, and others, which mediate inflammatory reactions, causing the death of immunogenic cells, a decrease in the number of Tregs and stimulating the expansion of antitumor cells [39,40]. Moreover, low doses of alkylating cytostatics, and melphalan in particular, are even more capable of depleting the CD4+CD25+Treg cycle and suppressing their suppressive activity, further increasing the possibility of enhancing the immune response and aggravating the inflammatory response [20,21]. In COVID-19, this is fraught with deterioration and death. However, when using low doses of cytostatics, RI deterioration was not observed; on the contrary, a positive effect is declared, which contradicts the generally accepted paradigm, emphasizing the discrepancy between the expected and declared results. Therefore, of course, in this case, the question cannot but arise about the same positive result when using opposite methods and approaches to influencing Treg, based on the concepts of IR and corresponding to the generally accepted paradigm, which differ significantly from each other in the features of their pathogenetic effect on the processes of not only inflammation, but also tumor development, as shown above, which, naturally, a priori suggests a different result. Firstly, perhaps this will be explained by the ultra-low dose used, in connection with which we will dwell on somewhat similar terms, definitions—"low doses", "ultra-low doses", and "homeopathic doses". It should be noted immediately that these terms, are closely related in meaning, as they imply the use of doses that differ significantly from the so-called effective doses used in clinical practice according to established pharmacological principles. All of them differ in that the doses are an order of magnitude or more lower than established, starting from 1/1000. At the same time, the meaning of their use also differs significantly. Thus, "homeopathic doses" are characterized by the fact that a substance is used that causes a reaction with subsequent pathological changes, but in ultra-low doses due to significant dilution, not accompanied by such reactions; however, it subsequently modifies the responses to doses approaching those that previously caused pathology. The effect of "ultra-low doses" is that their use eliminates the side effects characteristic of some drugs with a therapeutic effect. A decrease in the dose of the latter first negates the effect of the drug itself, and then its further reduction is accompanied by a therapeutic effect that exceeds the original. Similar to the last option, the use of "ultra-low doses" in the case of IR. In their opinion, the reduction of the active substance helps to eliminate side effects and modify its cytotoxic effect into an immunomodulatory one, which causes a blockade of IL-2 and a decrease in Treg. However, this runs counter to the goal of treatment, which is to relieve inflammation, and contributes to the effect described above: depletion of the CD4+CD25+Treg cycle and inhibition of their suppressor activity, thereby increasing the possibility of enhancing the immune response and aggravating the inflammatory reaction [20,31]. At the same time, it is quite obvious that here too, there is a discrepancy between the expected effect of the paradigm and the results of a large number of studies, as declared by the IR. It is appropriate to draw an analogy between the declared effects of homeopathy (but, in fact, absent due to the ultra-low doses of substances affecting the body, as a result of which homeopathy is officially recognized in many countries, including Russia and the USA, as pseudoscience) and the declared effects of ultra-low doses when using cytostatics, indicating a discrepancy between the expected (according to the existing paradigm) reaction in the form of an exacerbation, but declared in the form of a positive effect, which, according to the existing paradigm, is not expected. Secondly, considering the regular references of the IRI to their previous works as fundamental and shaping their subsequent views on the problem of Treg and inflammation, we conducted a retrospective analysis of the studies based on these works. We will not dwell on it in detail, but will only note that these studies include a number of significant shortcomings of various kinds and will refer the interested reader to previous works, in which they are largely reflected [29,41,42]. The latter are especially obvious in conditions when the effect of, for example, IL-2 depends on its levels and the role of the clinical part of the study and interpretation of its results becomes even more significant, including the adequacy of methodological features, the presence of a share of objective research methods and assessment in the delivery of the drugs used, their effects and interpretation of the doses used and other factors that have various shortcomings in studies on IR. For example, there is a subjective assessment of the physical properties of the respiratory tract mucosa, which is often far from objective indicators; there is no standardization of collecting material for morphological assessment; and there are no declared objective methods for assessing the regenerative processes underlying the declared IR results. Unlike other researchers, they did not use any of the factors that sig-

nificantly affect the deposition of the inhalant, on which the effect of its action significantly depends; however, they obtained a similar positive and even predominant effect, which is reflected in a number of publications [20,21]. At the same time, the controversial references of IR to the "palliatropic" effect and the possibility of environmental influence to explain the mechanism of action of alkylating cytostatics are not relevant, relating in the first case to the field of thermonuclear physics, and in the second, to the absence of such data in the literature. Undoubtedly, one cannot ignore the fact that from the position of the carcinogenic effect, alkylating cytostatics are included in the first, most significant group in this regard, and low harmless doses, according to experts in this field, do not exist for this group of drugs and their safety from this position according to existing standards has not been proven [43]. Regarding the effect on Treg in the tumor process, it is important to emphasize that the modern approach to cancer immunotherapy, in contrast to the treatment of inflammation, which requires the growth of Treg and the suppression of inflammation, consists, on the contrary, in the activation of immune reactions by depleting Treg and expressing Teff, which leads to an increase in the immune response and an increase in antitumor activity. The obtained data suggest that Treg cell depletion is directly proportional to antitumor activity and inversely proportional to tumor progression [44]. This is the basis for targeted approaches that involve influencing Tregs in cancer therapy by depleting them using various drugs and methods. Thus, some authors achieve this by using alkylating drugs that have an immunostimulatory effect due to Treg depletion, which can be enhanced by administering low doses and blocking Il-2R. Others act on CD25 using monoclonal antibodies or by blocking CTLA-4 with monoclonal antibodies, a molecule expressed by Treg that can kill effector Tregs or weaken their suppressive activity, contributing to a significant increase in immunotherapy in cancer [32]. However, systemic depletion of Treg cells can simultaneously cause pathogenic autoimmunity. One approach to creating effective antitumor immunity without autoimmunity is to target terminally differentiated Tefs, rather than all FOXP3 + T cells, since Tefs are the predominant cell type in tumor tissues [45]. Therefore, Treg depletion is increasingly considered as an adjuvant therapy in the development of an anticancer vaccine.

It should be noted separately that a significant positive role of Treg in the processes of restoring and regenerating lung tissue has been established, given its potential in this regard [46]. However, due to the capacity of the material, this requires further reflection in a separate work.

Considering the problem of modulating therapeutic effects on Tregs, it is necessary to emphasize that it is inextricably linked with the delivery of agents that can be used in this regard, as a result of which we will briefly dwell on this.

5. Delivery of Agents for Modulating Action on Treg, Use and Optimization of the Direct, Inhalation, Method

It is known that the effect on the lungs can be carried out systemically by parenteral administration of therapeutic agents, as well as by direct administration to the respiratory tract in the form of aerosols. Inhalation of drugs provides direct delivery to the site of inflammation in the lungs, potentially maximizing their therapeutic effect and minimizing systemic side effects. At the same time, various aerosol technologies are being developed that enable the delivery of drugs to target areas of the lungs where the pathological process or structures, key pathogenetic links requiring intervention, are localized. As is known, the delivery of drugs in the form of an aerosol directly to the lungs allows for bypassing biological barriers, particularly the gastrointestinal tract, which inactivates the action of pharmacological drugs, which is a clear advantage of aerosol therapy (AT). Thus, AT helps reduce the effective dose of pharmacological drugs and their side effects, while increasing the effectiveness of their use [47–49]. These aspects also concern the effect on Tregs, which are localized mainly in the distal parts of the lungs, characterized by the most problematic achievement of them due to the functioning of the protective mechanisms of the respiratory organs, including physical (aerodynamic filtration, mucociliary and cough clearances), as well as immunological (alveolar clearance) [50].

Recently, significant progress has been made in developing technical and therapeutic means for aerosol therapy. At the same time, AT is still underused and more often pulmonologists and especially general practitioners use parenteral routes of administration, given that the basic foundations of AT, without which its effectiveness may be nil, are at the junction of different sciences and do not take into account the possibilities regarding the use of the influence of the described factors on aerosol deposition, on which the effectiveness of AT largely depends. The

methods of AT, as outlined in various methodological recommendations, reference books, monographs, and other literary sources, generally do not specifically reflect the inhalation modes and their parameters for optimal implementation. Inhalation is typically provided in a single key, constant throughout its entire duration. Thus, the potential opportunity for targeted deposition of medicinal substances in the lungs is not utilized, considering the location of their optimal effect, the degree of prevalence of the pathological process, and its localization. This reduces the effectiveness of AT, increases the amount of the drug used, and prolongs the treatment period, which is a significant drawback. It should be noted that AT, against the backdrop of scientific and technical progress, has undergone evolutionary development, resulting in several methods and approaches that enable the mitigation of this drawback and the realization of its advantages in various areas. They were mainly based on considering several key components of the AT and the factors influencing them, including the aerodynamics of the AP. the kinetics of aerosol flight, the physicochemical properties of aerosols, and the technical capabilities of inhalation equipment. This can be seen in the example of inhalation delivery of different groups of immunosuppressants used to optimize the treatment of inflammation in the AP by suppressing it. Thus, some authors ensured the transfer of immunosuppressants, such as cytostatics with an antiproliferative effect or immunosuppressants that affect immunophilins, from a hydrophobic state unsuitable for AT due to their low bioavailability to a more optimal state for aerosol action, a hydrophilic state [51]. That is, it was taken into account that any drug delivered to the lungs must have appropriate bioavailability due to its hydrophilicity and lipophilicity, in order to dissolve well in the mucous layer and be absorbed through cell membranes. To improve solubility in water, they form a complex, in particular cyclosporine, with cyclodextrin, which was carried out under the control of the viscosity and osmotic pressure of the resulting solution, as well as the nature of the movement of the cilia under a microscope, to exclude possible changes in this case. To solve this challenging problem, additional solubilizers were also employed, including propylene glycol or ethanol [52]. However, as is known, they can have an irritating effect on the mucous link of the respiratory tract and, by increasing its osmolarity, promote hypersecretion of respiratory tract mucus, and also suppress the activity of the cilia of the mucociliary apparatus of the bronchi, disrupting muciliary clearance, which are an important pathogenetic link in lung diseases, which requires its correction [52–54]. Therefore, the next step in improving this aerosol technology was the use of other, more optimal water-based inhalant formulations, including liposomes or other dispersions [55]. Other researchers have improved drug delivery by using nanocarriers. The latter have many advantages through the inhalation route, favoring the degree of penetration and uniform distribution of the drug in the respiratory sections of the AP, its better solubilization, and prolonged release, and action, which contribute to a decrease in the frequency of dosing, as well as better patient compliance, reduced side effects and improved internalization of the drug into cells [56]. In this case, excess hydration in the alveoli is excluded, which is a significant pathogenetic link in the pneumonic process, especially in acute viral diseases, including SARS-CoV-2. The effectiveness of inhaled drugs can also be increased by optimizing the deposition of the inhalant in the respiratory tract, due to preliminary medicinal improvements in their patency and/or a specific inhalation mode based on the patient's breathing parameters and the inhaler operation [57]. Several researchers utilized the inhaler's operation mode, which is based on the air flow rate parameters set by the inhaler [58]. The breathing mode, for example, its 3–10-second delay, was also used as an important factor for optimizing AT [59,60]. Naturally, technologies that take into account a set of factors determining the effectiveness of AT had an advantage. All these improvements in AT made it possible to significantly reduce the effective doses of these drugs and increase their therapeutic effect, both by optimizing the action of the drugs themselves and by significantly reducing their toxic properties, thereby leveling or even eliminating the side effects of their use. Accordingly, this helps to reduce the effective dose of corticosteroids and their systemic side effects. In addition, it is quite obvious that a simple linear reduction in the dose of drugs by simply diluting them with saline, as was the case in some studies [21,61], without taking into account the factors affecting their pulmonary deposition, significantly reduces or eliminates the effectiveness of the targeted action of the drugs in question. The use of a metered-dose jet (compressor type) inhaler with one sufficiently high air flow rate, up to 5 l/min or more, which is set independently of the patient's breathing pattern at an operating pressure of 1.4 bar increases the air flow rate in the respiratory tract and its turbulence, promoting even more proximal deposition and reducing penetration into the distal parts of the lungs, which are the target when influencing Treg. In addition, the dose of the inhaled drug is reduced not only by a 100-fold decrease in the initial dilution but also by an additional involuntary decrease of approximately 10 times, which occurs when delivering the drug by inhalation [62,63]. Only a comprehensive approach that takes into account the above factors allows us to achieve, but only to a certain extent,

the possibility of reducing the effective dose of drugs, along with a decrease in side effects and an increase in the effectiveness of AT. We, like several other authors, also considered that the therapeutic effect of drugs largely depends on their deposition site in the respiratory tract [64]. The latter, as is known, is largely determined by the size of inhaled particles and the speed of air flow, which are influenced by various factors, including the breathing mode and the operation of the inhaler [65, 66]. Taking these aspects into account, using a direct assessment using the radioaerosol method with the use of radioalbumin microspheres labeled with Tc 99m, we developed a new method of AT [67], which significantly increases the effectiveness of the prevention and treatment of lung diseases. It involves inhalation in a two-stage mode to ensure the possibility of targeted distribution of medicinal substances in specified sections of the lungs, taking into account the prevalence and localization of the pathological process both throughout the tracheobronchial tree (e.g., in chronic bronchitis) and in its central (e.g., in acute tracheobronchitis) or peripheral sections (e.g., in acute pneumonia, bronchiolitis). In this case, the RR is 9±2 per min., DR — 500±25 ml and SVPZI — 3 l/min, in its first half (mode I) and 30±2 per min., 175±25 ml and 21 l/min, respectively, in the second half (mode II) for uniform distribution of medicinal substances throughout the respiratory tract; inhalation mode I is used to deposit the inhalant in the peripheral sections, and mode II in the central ones. This method was tested during AT in 345 patients with various bronchopulmonary diseases, for whom it was indicated, and included expectorants, hormonal drugs, bronchodilators, and antibiotics. The results obtained were compared with the effect of inhalations carried out according to the generally accepted method. As the leading criterion for the comparative assessment of equally positive outcomes of therapy, we used the bed-day. The results showed that inhalation using this method reliably reduced the duration of treatment for patients, indicating a significant increase in the effectiveness of AT for both acute and chronic diseases of the respiratory system [67]. It should be noted that the applied breathing regime is quickly and easily assimilated according to the specified parameters and is stably reproduced subsequently, which is consistent with the results of studies on breathing regulation [68]. Moreover, it is interesting that patients with more pronounced bronchial patency disorders tend to be more flexible in this regard. Carrying out inhalation in a given breathing mode, which is practiced the day before inhalation and can be controlled during its implementation, does not require complex equipment and large economic costs and can be carried out in almost any conditions where only inhalation equipment is available.

Considering the localization of Tregs mainly in the distal respiratory tract, it is necessary to carry out inhalation with breathing parameters and an inhaler operating mode that ensure this localization. With normal bronchial patency, this is achieved by using deep and slow breathing to ensure laminar airflow to the distal bronchi, holding it for several seconds on inhalation to maximize sedimentation due to sedimentation processes. However, in pathology, various obstructive ventilation disorders may occur, caused by different reasons, as reflected in **Figure 5**. Bronchial obstruction, causing an increase in airflow velocity and the occurrence/enhancement of its turbulence, contributes to a more central distribution of the inhaled drug and decreased deposition in the peripheral region of the lung, which, according to the researchers studying this problem, is a target area for Treg [69]. Leveling the effect of obstruction is not an easy task, and to reduce it to a certain extent, it is necessary to include medicated preparations for the respiratory tract into inhalation technology. For this purpose, the effect of mucoactive agents is intensified to enhance the evacuation of bronchial contents, as well as to provide anti-inflammatory and bronchodilator effects. Breathing is increased, if possible, to the deepest and slowest (the target parameters are reflected above in the two-stage mode); the air flow velocity set by the inhaler is set to a minimum. If possible, a smaller particle size is used, up to 1–2 μm, which can be achieved initially or by using a sieve.

6. Conclusion

Thus, Treg, being an important link in the immune system, provides its control and the balance necessary for its proper functioning. According to the generally accepted paradigm, their main function is to limit inflammation, which is characteristic of diseases affecting organs and systems, particularly the lungs. Various effects on Treg can have a modulating effect on the processes of inflammation and carcinogenesis. Strengthening Treg is having an ambivalent impact—it suppresses Tef and excessive immune inflammatory response, but at the same time, the antitumor immune response is suppressed. Reducing Treg promotes Tef expression, initiation of inflammation and autoimmune reactions, but significantly increases the activity of antitumor cells. Innovative methods for treating inflammation and carcinogenesis aim to modulate Treg function, which is achieved in different ways. Strengthening Tregs to reduce inflammation. This is achieved by direct introduction of Tregs into the human body by their adoptive

transfer systemically or directly into the target organ, for example, into the lungs in the form of an aerosol, which is more optimal, especially when using specified inhalation modes to control the deposition of the inhalant, on which the effectiveness of aerosol therapy depends. Alternatively, this can be achieved indirectly through the same pathways, affecting the key link of Tregs by activating small doses of its subordinate component, IL-2, on which the functional activity of Tregs depends. The use by IR of effects that selectively deplete Tregs for the treatment of inflammation, including blocking IL-2 due to the use of ultra-low doses of alkylating drugs, does not align with the generally accepted paradigm and is inappropriate, as it is associated with side effects.

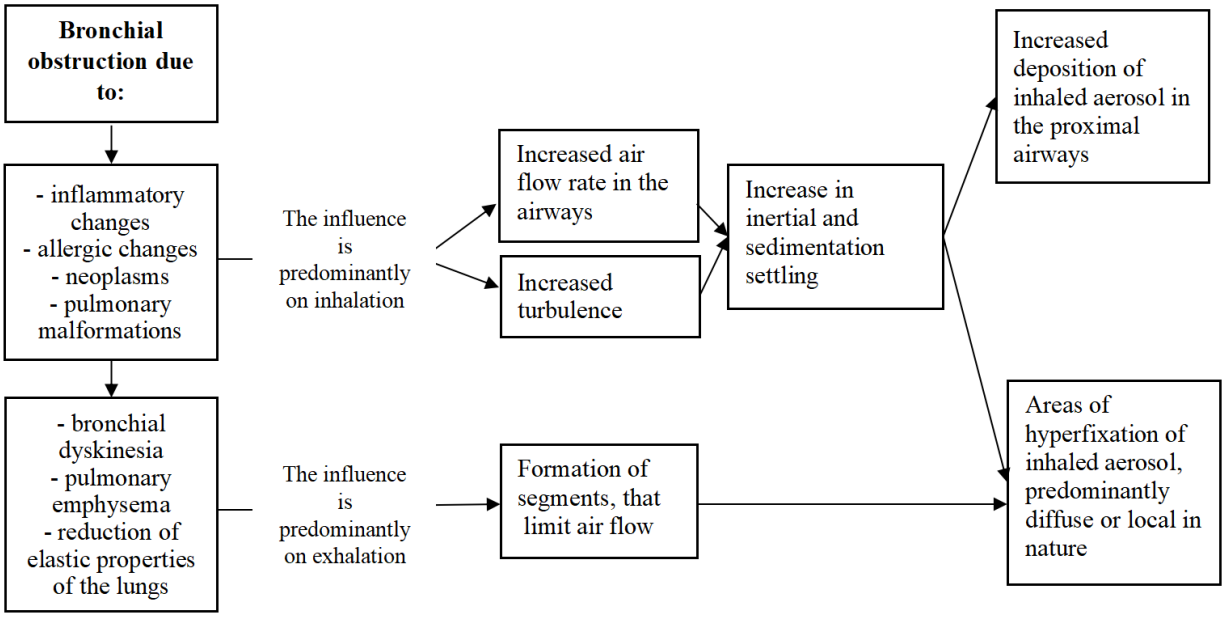

Figure 5. The effect of bronchial obstruction on inhalant deposition depending on the genesis of the pathology and respiratory phases.

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Informed consent was not required, since the work was of a review analytical nature and did not require human participation.

Data Availability Statement

We declare that no data relating to our research and confirming the presented results, other than those given in the references regarding the past, have been created.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Ahmad, S.; Manzoor, S.; Siddiqui, S.; et al. Epigenetic Underpinnings of Inflammation: Connecting the Dots Between Pulmonary Diseases, Lung Cancer and COVID-19. *Semin. Cancer Biol.* **2022**, *83*, 384–398. [CrossRef]
2. Fuchs, A.; Gliwiński, M.; Grageda, N.; et al. Minimum Information About T Regulatory Cells: A Step Toward Reproducibility and Standardization. *Front. Immunol.* **2018**, *8*, 1844. [CrossRef]
3. Ito, H.; Seishima, M. Regulation of the Induction and Function of Cytotoxic T Lymphocytes by Natural Killer T Cell. *J. Biomed. Biotechnol.* **2010**, *2010*, 641757. [CrossRef]
4. Chen, S.; Saeed, A.F.U.H.; Liu, Q.; et al. Macrophages in Immunoregulation and Therapeutics. *Signal Transduct. Target Ther.* **2023**, *8*, 207. [CrossRef]
5. Zhang, Y.S.; Chen, Y.Q. Dysfunctional Regulatory T Cell: May Be an Obstacle to Immunotherapy in Cardiovascular Diseases. *Biomed. Pharmacother.* **2024**, *173*, 116359. [CrossRef]
6. Rajendeeran, A.; Tenbrock, K. Regulatory T Cell Function in Autoimmune Disease. *J. Transl. Autoimmun.* **2020**, *4*, 100130. [CrossRef]
7. Mock, J.R.; Dial, C.F.; Tune, M.K.; et al. Impact of Regulatory T Cells on Type 2 Alveolar Epithelial Cell Transcriptomes During Resolution of Acute Lung Injury and Contributions of IFN-γ. *Am. J. Respir. Cell Mol. Biol.* **2020**, *63*, 464–477. [CrossRef]
8. Liang, J.; Bi, G.; Shan, G.; et al. Tumor-Associated Regulatory T Cells in Non-Small-Cell Lung Cancer: Current Advances and Future Perspectives. *J. Immunol. Res.* **2022**, *2022*, 4355386. [CrossRef]
9. Principe, D.R.; Chiec, L.; Mohindra, N.A.; et al. Regulatory T-Cells as an Emerging Barrier to Immune Checkpoint Inhibition in Lung Cancer. *Front. Oncol.* **2021**, *11*, 684098. [CrossRef]
10. Wang, H.; Wang, Z.; Cao, W.; et al. Regulatory T Cells in COVID-19. *Aging Dis.* **2021**, *12*, 1545–1553. [CrossRef]
11. Xu, Z.; Jiang, X.; Dai, X.; et al. The Dynamic Role of FOXP3+ Tregs and Their Potential Therapeutic Applications During SARS-CoV-2 Infection. *Front. Immunol.* **2022**, *13*, 916411. [CrossRef]
12. Zhang, R.; Zhao, Y.; Chen, X.; et al. Low-Dose IL-2 Therapy in Autoimmune Diseases: An Update Review. *Int. Rev. Immunol.* **2024**, *43*, 113–137. [CrossRef]
13. Allegretti, J.R.; Mitsialis, V.; Canavan, J.B.; et al. Low-Dose Interleukin 2 for the Treatment of Moderate to Severe Ulcerative Colitis. *Gastroenterology* **2023**, *165*, 492–495. [CrossRef]
14. Hoyer, K.K.; Dooms, H.; Barron, L.; et al. Interleukin-2 in the Development and Control of Inflammatory Disease. *Immunol. Rev.* **2008**, *226*, 19–28. [CrossRef]
15. Yu, A.; Snowhite, I.; Vendrame, F.; et al. Selective IL-2 Responsiveness of Regulatory T Cells Through Multiple Intrinsic Mechanisms Supports the Use of Low-Dose IL-2 Therapy in Type 1 Diabetes. *Diabetes* **2015**, *64*, 2172–2183. [CrossRef]
16. Veiga-Parga, T.; Sehrawat, S.; Rouse, B.T. Role of Regulatory T Cells During Virus Infection. *Immunol. Rev.* **2013**, *255*, 182–196. [CrossRef]
17. Wang, F.; Hou, H.; Luo, Y.; et al. The Laboratory Tests and Immunity of COVID-19 Patients With Different Severity of Illness. *JCI Insight* **2020**, *5*, e137799. [CrossRef]
18. Dicharry, D.; Malek, A.E. Unlocking the Role of Treg Cells Immune Response and Infectious Risk Following CAR T-Cell Therapy in Patients With Cancer. *Int. J. Mol. Sci.* **2025**, *26*, 1602. [CrossRef]
19. Li, J.; Chen, X.; Qu, Y. Effects of Cyclophosphamide Combined With Prednisone on TNF-α Expression in Treatment of Patients With Interstitial Lung Disease. *Exp. Ther. Med.* **2019**, *18*, 4443–4449. [CrossRef]
20. Pukhalsky, A.L.; Shmarina, G.V.; Aleshkin, V.A. Immunological Disorders and Cognitive Deficits During Stress and Physiological Aging. Part II: New Approaches to the Prevention and Treatment of Cognitive Disorders. *Bull. Russ. Acad. Med. Sci.* **2014**, 30–37. (in Russian)
21. Sinitsyn, E.A.; Zykova, A.A.; Shamin, R.V.; et al. Efficacy and Safety of Ultra-Low-Dose Inhaled Melphalan in the Treatment of Hospitalized Patients With COVID-19. *Acta Biomed. Sci.* **2022**, *7*, 12–23. (in Russian)
22. Shmarina, G.; Pukhalsky, A.; Alioshkin, V.; et al. Melphalan Reduces the Severity of Experimental Colitis in Mice by Blocking Tumor Necrosis Factor-Alpha Signaling Pathway. *Ann. N. Y. Acad. Sci.* **2007**, *1096*, 97–105. [CrossRef]
23. Pukhalsky, A.; Shmarina, G.; Alioshkin, V.; et al. Alkylating Drugs Applied in Non-Cytotoxic Doses as Novel Compounds Targeting Inflammatory Signal Pathway. *Biochem. Pharmacol.* **2006**, *72*, 1432–1438. [CrossRef]
24. Fan, M.Y.; Low, J.S.; Tanimine, N.; et al. Differential Roles of IL-2 Signaling in Developing Versus Mature Tregs. *Cell Rep.* **2018**, *25*, 1204–1213. [CrossRef]
25. Raeber, M.E.; Sahin, D.; Karakus, U.; et al. A Systematic Review of Interleukin-2-Based Immunotherapies in Clinical Trials for Cancer and Autoimmune Diseases. *EBioMedicine* **2023**, *90*, 104539. [CrossRef]
26. Zhang, J.; Huang, Y. Low-Dose IL-2 in the Treatment of Immune-Related Diseases. *Eur. J. Inflamm.* **2021**, *19*,

20587392211039935. [CrossRef]
27. Amini, L.; Kaeda, J.; Weber, O.; et al. Low-Dose Interleukin-2 Therapy: Fine-Tuning Treg in Solid Organ Transplantation? *Transplantation* **2024**, *108*, 0000000000004866. [CrossRef]
28. Ghelani, A.; Bates, D.; Conner, K.; et al. Defining the Threshold IL-2 Signal Required for Induction of Selective Treg Cell Responses Using Engineered IL-2 Muteins. *Front. Immunol.* **2020**, *11*, 1106. [CrossRef]
29. Kobylyansky, V.I. Assessing the Effectiveness of Inhaled Immunosuppressive Drugs in the Treatment of COVID-19. *Infect. Dis. News Opin. Train.* **2023**, *12*, 81–89. (in Russian)
30. Lutsiak, M.E.; Semnani, R.T.; De Pascalis, R.; et al. Inhibition of $CD4^+25^+$ T Regulatory Cell Function Implicated in Enhanced Immune Response by Low-Dose Cyclophosphamide. *Blood* **2005**, *105*, 2862–2868. [CrossRef]
31. Su, Y.C.; Rolph, M.S.; Cooley, M.A.; et al. Cyclophosphamide Augments Inflammation by Reducing Immunosuppression in a Mouse Model of Allergic Airway Disease. *J. Allergy Clin. Immunol.* **2006**, *117*, 635–641. [CrossRef]
32. Flores, C.; Fouquet, G.; Cruz Moura, I.; et al. Lessons to Learn From Low-Dose Cyclosporin-A: A New Approach for Unexpected Clinical Applications. *Front. Immunol.* **2019**, *10*, 588. [CrossRef]
33. Loeffler, M.; Krüger, J.A.; Reisfeld, R.A. Immunostimulatory Effects of Low-Dose Cyclophosphamide Are Controlled by Inducible Nitric Oxide Synthase. *Cancer Res.* **2005**, *65*, 5027–5030. [CrossRef]
34. Becker, J.C.; Schrama, D. The Dark Side of Cyclophosphamide: Cyclophosphamide-Mediated Ablation of Regulatory T Cells. *J. Invest. Dermatol.* **2013**, *133*, 1462–1465. [CrossRef]
35. Scurr, M.; Pembroke, T.; Bloom, A.; et al. Low-Dose Cyclophosphamide Induces Antitumor T-Cell Responses, Which Associate With Survival in Metastatic Colorectal Cancer. *Clin. Cancer Res.* **2017**, *23*, 6771–6780. [CrossRef]
36. Heylmann, D.; Bauer, M.; Becker, H.; et al. Human $CD4^+CD25^+$ Regulatory T Cells Are Sensitive to Low-Dose Cyclophosphamide: Implications for the Immune Response. *PLoS One* **2013**, *8*, e83384. [CrossRef]
37. Peng, K.; Li, X.; Yang, D.; et al. Risk of Autoimmune Diseases Following COVID-19 and the Potential Protective Effect From Vaccination: A Population-Based Cohort Study. *EClinicalMedicine* **2023**, *63*, 102154. [CrossRef]
38. Sharma, C.; Bayry, J. High Risk of Autoimmune Diseases After COVID-19. *Nat. Rev. Rheumatol.* **2023**, *19*, 399–400. [CrossRef]
39. Caraffa, A.; Gallenga, C.E.; Kritas, S.K.; et al. CAR-T Cell Therapy Causes Inflammation by IL-1 Which Activates Inflammatory Cytokine Mast Cells: Anti-Inflammatory Role of IL-37. *J. Biol. Regul. Homeost. Agents* **2019**, *33*, 1981–1985.
40. Kuczma, M.; Ding, Z.C.; Zhou, G. Immunostimulatory Effects of Melphalan and Usefulness in Adoptive Cell Therapy With Antitumor $CD4^+$ T Cells. *Crit. Rev. Immunol.* **2016**, *36*, 179–191. [CrossRef]
41. Revannasiddaiah, S.; Devadas, S.K.; Palassery, R.; et al. A Potential Role for Cyclophosphamide in the Mitigation of Acute Respiratory Distress Syndrome Among Patients With SARS-CoV-2. *Med. Hypotheses* **2020**, *144*, 109850. [CrossRef]
42. Kobylyansky, V.I. Possibilities of Using Immunosuppressants and Their Aerosol Delivery to the Lungs in the Treatment of Severe Bronchial Asthma. *Clin. Pharmacol. Ther.* **2022**, *31*, 14–19. (in Russian)
43. Tyulyandin, S.A.; Samoilenko, I.V.; Izmerova, N.I. *Guidance for Medical Personnel on the Safe Handling of Anticancer Drugs*; Research Institute of Occupational Medicine RAS: Moscow, Russia, 2012. (in Russian)
44. Zeng, G.; Jin, L.; Ying, Q.; et al. Regulatory T Cells in Cancer Immunotherapy: Basic Research Outcomes and Clinical Directions. *Cancer Manag. Res.* **2020**, *12*, 10411–10421. [CrossRef]
45. Tanaka, A.; Sakaguchi, S. Regulatory T Cells in Cancer Immunotherapy. *Cell Res.* **2017**, *27*, 109–118. [CrossRef]
46. Kotton, D.N.; Morrisey, E.E. Lung Regeneration: Mechanisms, Applications and Emerging Stem Cell Populations. *Nat. Med.* **2014**, *20*, 822–832. [CrossRef]
47. Kohler, D. Aerosols for Systemic Treatment. *Lung* **1990**, *168*, 677–684. [CrossRef]
48. Lai, S.; Hard, D.M.; Bhalla, K.K.; et al. Comparison of Beclometasone Dipropionate Aerosol and Prednisolon in Reversible Airways Obstruction. *Br. Med. J.* **1972**, *3*, 314–320. [CrossRef]
49. Plit, M.; Goldman, H.I.; Cassel, M.L.; et al. The Bronchodilator Action of Fenoterol (Berotec) in Asthma. *Med. Proc.* **1972**, *18*, 4.
50. Kobylyansky, V.I. *Mucociliary System; Fundamental and Applied Aspects*; BINOM: Moscow, Russia, 2008. (in Russian)
51. Fukava, H.; Limura, A.; Hoshiko, K.; et al. A Cyclosporin A/Maltosyl-α-Cyclodextrin Complex for Inhalation Therapy of Asthma. *Eur. Respir. J.* **2003**, *22*, 213–219. [CrossRef]
52. Yuan, Y.; Che, X.; Zhao, M. Development of Cyclosporine A Microemulsion for Parenteral Delivery. *J. Microencapsul.* **2015**, *32*, 273–280. [CrossRef]
53. European Patent Office. Cyclosporine Formulations for Use in the Prevention or Treatment of Pulmonary

Chronic Graft Rejection. *Eur. Pat. Appl.* EP 3069711A1, 15 March 2015.
54. Bustamante-Marin, X.M.; Ostrowski, L.E. Cilia and Mucociliary Clearance. *Cold Spring Harb. Perspect. Biol.* **2017**, *9*, a028241. [CrossRef]
55. Trammer, B.; Amann, A.; Haltner-Ukomadu, E.; et al. Comparative Permeability and Diffusion Kinetics of Cyclosporine A Liposomes and Propylene Glycol Solution From Human Lung Tissue Into Human Blood ex Vivo. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 758–764. [CrossRef]
56. Ahmad, J.; Akhter, S.; Rizwanullah, M.; et al. Nanotechnology-Based Inhalation Treatments for Lung Cancer: State of the Art. *Nanotechnol. Sci. Appl.* **2015**, *8*, 55–66. [CrossRef]
57. Labiris, N.R.; Dolovich, M.B. Pulmonary Drug Delivery. Part I: Physiological Factors Affecting Therapeutic Effectiveness of Aerosolized Medications. *Br. J. Clin. Pharmacol.* **2003**, *56*, 588–599. [CrossRef]
58. Labiris, N.R.; Dolovich, M.B. Pulmonary Drug Delivery. Part II: The Role of Inhalant Delivery Devices and Drug Formulations in Therapeutic Effectiveness of Aerosolized Medications. *Br. J. Clin. Pharmacol.* **2003**, *56*, 600–612. [CrossRef]
59. An, B.; Reinhardt, R.R. Effects of Different Durations of Breath Holding After Inhalation of Insulin Using the AERx Insulin Diabetes Management System. *Clin. Ther.* **2003**, *25*, 2233–2244. [CrossRef]
60. Ahookhosh, K.; Saidi, M.; Mohammadpourfard, M.; et al. Flow Structure and Particle Deposition Analyses for Optimization of a Pressurized Metered Dose Inhaler (pMDI) in a Model of Tracheobronchial Airway. *Eur. J. Pharm. Sci.* **2021**, *164*, 105911. [CrossRef]
61. Zykov, K.A. Method of Treatment of Patients with Bronchial Asthma [Bulletin No. 15; Issue No. 2356574]. 2008.
62. Newman, S.P.; Clarke, S.W. Inhalation Devices and Techniques. In *Asthma*, Clark, T.J.H.; Godfrey, S.; Lee, T.H., Eds.; Chapman and Hall: London, UK, 1992; pp. 469–505.
63. Tena, A.F.; Clarà, P.C. Deposition of Inhaled Particles in the Lungs. *Arch. Bronconeumol.* **2012**, *48*, 240–246. [CrossRef]
64. Chung, K.F.; Jeyasingh, K.; Snashall, P.D. Influence of Airway Calibre on the Intrapulmonary Dose and Distribution of Inhaled Aerosol in Normal and Asthmatic Subjects. *Eur. Respir. J.* **1988**, *1*, 890–895. [CrossRef]
65. Heyder, J. Mechanisms of Aerosol Particle Deposition. *Chest* **1980**, *80*, 820–824. [CrossRef]
66. Morgan, W.K.C.; Ahmad, D.; Chamberlain, M.Y.; et al. The Effect of Exercise on an Inhaled Aerosol. *Respir. Physiol.* **1984**, *56*, 327–338. [CrossRef]
67. Kobilyansky, V.I.; Artyushkin, A.V. USSR Author's Certificate for Invention No. 1524904. 1989. (in Russian)
68. Breslav, I.S. *Breathing Patterns*; Nauka: Leningrad, Russia, 1984. (in Russian)
69. Zhang, S.; Gang, X.; Yang, S.; et al. The Alterations in and the Role of the Th17/Treg Balance in Metabolic Diseases. *Front. Immunol.* **2021**, *12*, 678355. [CrossRef]

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