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A System Mathematical Model of Cell-cell Communication Network in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a devastating and chronic neurodegenerative disease without any known cure. In the brain and spinal cord of both patients and animal models with ALS, neuroinflammation is a prominent pathological hallmark which is characterized by infiltrating T cells at sites of motor neurons injury. Their presence in mutant Cu²⁺/Zn²⁺ superoxide dismutase (mSOD1) induced ALS plays an important role in shifting the response of microglia from neuroprotective into neurotoxic. In order to better understand how these cells and their communication network collectively modulate the disease progress, we have established a mathematical model integrating diverse cells and cytokines. According to the experimental data sets, we first refined this model by identifying a link between TGF β and M1 microglia which can produce an optimized model to fit data sets better. Then based on this model, parameters were estimated using genetic algorithm. Sensitivity analysis of these parameters identified several factors such as release rate of IFN γ by T helper 1 (Th1) cells, which may be related to the heterogeneity between the patients with different survival time. Furthermore, the tests on T cell based therapeutic strategies indicated that elimination of Th1 cells is the most effective approach extending survival time. This confirmed the dominant role of Th1 cells in leading the rapid disorder in the later stage of ALS. For the therapies targeting cytokines, injection of IL6 can essentially augment the neuroprotective response and extend the life effectively by elevating the level of IL4, a neuroprotective cytokine, while direct injected IL4 will decay rapidly in ALS microenvironment and cannot provide a persistent protective effect. On the other hand, in spite of the attractive effect of direct elimination of mSOD1 or self-antigen, it is difficult to implement in CNS. As an alternative, elimination of IFN γ can be chosen as another effective therapy. In the future, if we combine the side effect of different therapies, this model can be used to optimize the therapeutic strategies so that they can effectively improve survival rates and quality of life for patients with ALS.

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is an adult-onset, rapidly progressive disorder characterized by selective degeneration of upper and lower motor neurons. The disease will result in various degrees of weakness and atrophy of limb musculature, spasticity, dysarthria (slurred speech), and dysphagia (difficulty swallowing), and death [1, 2]. At present, therapies for ALS are mainly symptomatic, and fail to halt disease progression. According to the ALS association, life expectancy after diagnosis can be from two to five years and about 20% of patients with ALS can live five years or more.

Although the pathogenesis of ALS is not clearly understood, an important step toward determining the cause came in 1993 when scientists discovered that mutations in the gene that produces the Cu/Zn superoxide dismutase (SOD1) enzyme were associated with some cases (approximately 20%) of familial ALS [3, 4]. This enzyme is a powerful antioxidant that protects the body from damage caused by superoxide, a toxic free radical generated in the mitochondria during normal metabolism. However, mutant SOD1 (mSOD1) not only decreased enzymatic activity to destroy free radicals in some situations, but also stimulates the microglia through CD14 and Toll-like receptors to release the toxic superoxide [5, 6]. These free radicals can accumulate in central nervous system (CNS) and cause damage to both mitochondrial and nuclear DNA and proteins within the motor neurons.

Neuroinflammation is a prominent pathological feature of ALS disease, which is characterized by the expansion of activated microglia and infiltrating T cells at sites of brain and spinal cord motor neuron injury [7, 8]. Microglia, resident macrophages in CNS, display functional plasticity during activation, which involves changes in cell number, morphology, surface receptors, and production of growth factors and cytokines [9]. T-cell-derived cytokines play critical roles in the control of the microglia phenotype. For instance, the differentiation from resting microglia into classically activated microglia (M1 microglia) can be induced by interferon gamma ($\text{IFN}\gamma$), one of the most important cytokines produced by T helper 1 (Th1) cells, in the presence of lipopolysaccharide (LPS) [10, 11]. M1 microglia secretes increased proinflammatory cytokines and free radicals, which promote the degradation of motor neurons. On the contrary, representative T helper 2 (Th2) cytokines, such as interleukin 4 (IL-4), can convert resting microglia to an alternatively activated phenotype (M2 microglia). M2 microglia is characterized by enhancing the production of neurotrophic factors, such as insulin-like growth factor 1 (IGF-1), which plays important roles in cell maintenance and repair [12]. The communication between the infiltrating T cells and microglia by cytokines has been suggested to perform a dramatic effect on the progress of ALS disease [13, 14]. IL4-releasing regulatory T (Treg) cells can support the neuroprotective response in the early stage while Th1 cells promote the neurotoxic response in the later stage [15]. However, due to the inherent heterogeneity of the disease microenvironment and the complexity of the cell-cell communication network, it remains poorly understood at the system level how these cells and their communication network collectively modulate the disease progress. Thus, the mathematical model should be a feasible approach to provide some insight into this system before experiment examines.

In this work, we establish a mathematical model integrating the diverse populations of cells and cytokines involved in the ALS disease system. This integrated model explicitly includes concepts such as cell proliferation, differentiation and degradation, as well as communication through cytokines. After the parameters are refined by the *in vivo* experiment data form [15], the integrated model is used to seek several potential therapeutic strategies for ALS disease.

Methods

mSOD1-induced ALS Biology and System Model

Fig. 1 shows the schematic diagram of mSOD1-induced ALS which is used to develop the computational system model, capturing the information of primary cells and proteins. Although mSOD1 has been considered as the most common factor related to familial ALS, the cause of initializing the disease progression is not fully understood. According to the Knudson hypothesis [16], we assume that, due to accumulated mutations in cells and stimulation from environment, a part of normal motor neurons (nMN) which do not release mSOD1 is converted into mSOD1-producing motor neurons (mMN) at the onset of the disease. The released mSOD1 from mMN can promote the M1 microglia to produce

superoxide, nitric oxide and other neurotoxic cytokines which cause the degradation of motor neurons into debris. The debris can be captured by antigen-presenting cells (APC) and then presented to naive T helper (Th0) cells as CNS-self-antigen (Ag). Upon antigenic stimulation, Th0 cells will expand and differentiate into at least four functionally distinct subsets: Th1, Th2, Treg and Th17 cells [17]. Th1 cells will secrete IFN γ which can conduct the classic activation of resting microglia, while Th2 and Treg cells will release IL4 which can conduct the alternative activation of resting microglia. In the contrary to the neurotoxic response of microglia, M2 microglia has the capability of preventing the degradation of motor neurons by releasing several neuroprotective factors, such as IGF-1. The communication between innate and adaptive immune system will shift the balance of the microenvironment from neuroprotection into neurotoxicity during the disease progress. Thus, enhancement of neuroprotective response is a straightforward aim to extend the life span of patients with ALS.

While most links between cells and proteins were established directly based on the literatures, it is remarkable that the link between M1 microglia and TGF β was added why we were trying to fit our mathematic model to the experiment data from [15]. TGF β is a kind of anti-inflammatory cytokines, which can be released by several different cells, such Treg cell and M2 microglia in our system. However, according to the experiment data from [15], TGF β keeps increasing throughout the disease progression while Treg cell and M2 microglia decrease rapidly at the late stage of the disease. As a result, it is expected that there should be another kind of TGF β releasing cells whose number increase, at least not decrease, at the late stage of the disease. In our system model, we chose Th1 cell and M1 microglia as two potential source of TGF β and then tested which can produce an optimized fit to the experiment data using the method described below. Finally, the link between TGF β and M1 microglia was confirmed in this system model.

System Mathematical Model

Our mathematical model is based on a well-mixed species system and twenty entities are integrated in this model. We present the system network as a set of coupled ordinary differential equations (ODEs) in terms of population dynamics for each entity. The rate of change for each entity is expressed by the production, degradation, conversion and interactions with other entities.

The Th0 cells can be produced, degraded and converted into functionally distinct subsets. The change rate of Th0 can be expressed as

$$d[Th0]/dt = P_{Th0} - D_{Th0} \cdot [Th0] - C_{Th0} \cdot H^1([Ag]) \cdot H^1([IL12] + b \cdot [IFN\gamma] + c \cdot [IL4] + d \cdot [TGF\beta]) \cdot [Th0] \quad (1)$$

where P_{Th0} , D_{Th0} and C_{Th0} are the production rate, degradation rate and conversion rate of Th0 cells. $H^n([x])$ is the Hill function as follow

$$H^n([x]) = \frac{[x]^n}{K^n + [x]^n} \quad (2)$$

where $[x]$ is the concentration of entity x , K is the concentration producing half occupation and n is Hill coefficient describing cooperativity. $[Ag]$ represents the neuron debris caused by neurodegradation and it is necessary for stimulating Th0 cells to initiate the differentiation into functionally distinct subsets. Four kinds of cytokines, IL12, IFN γ , IL4 and TGF β , are considered as the factors to conduct the differentiation direction in this system model. IL12 and IFN γ can skew the differentiation into a Th1-type response and IL4 prefers the Th2-type differentiation while TGF β conducts the Th0 differentiation into Treg

or Th17 cells. Parameters b , c and d are the relative weights which represent the capability of conducting differentiation direction by IFN γ , IL4 and TGF β while compared to IL12.

The ODEs for the population dynamics of Th1, Th2 and Treg cells are similar with Th0 cells, which are listed as follow

$$\begin{aligned} d[Th1]/dt = & P_{Th1} - D_{Th1} \cdot [Th1] \\ & + C_{Th0} \cdot H^1([Ag]) \cdot H^1([IL12] + b \cdot [IFNr] + c \cdot [IL4] + d \cdot [TGFb]) \cdot [Th0] \cdot \frac{[IL12] + b \cdot [IFNr]}{[IL12] + b \cdot [IFNr] + c \cdot [IL4] + d \cdot [TGFb]} \cdot H^{-1} \end{aligned} \quad (3)$$

$$\begin{aligned} d[Th2]/dt = & P_{Th2} - D_{Th2} \cdot [Th2] \\ & + C_{Th0} \cdot H^1([Ag]) \cdot H^1([IL12] + b \cdot [IFNr] + c \cdot [IL4] + d \cdot [TGFb]) \cdot [Th0] \cdot \frac{c \cdot [IL4]}{[IL12] + b \cdot [IFNr] + c \cdot [IL4] + d \cdot [TGFb]} \cdot H^{-1} \end{aligned} \quad (4)$$

$$\begin{aligned} d[Treg]/dt = & P_{Treg} - D_{Treg} \cdot [Treg] \\ & + C_{Th0} \cdot H^1([Ag]) \cdot H^1([IL12] + b \cdot [IFNr] + c \cdot [IL4] + d \cdot [TGFb]) \cdot [Th0] \cdot \frac{d \cdot [TGFb]}{[IL12] + b \cdot [IFNr] + c \cdot [IL4] + d \cdot [TGFb]} \cdot (1 - V) \end{aligned} \quad (5)$$

where P_x and D_x are the production rate and degradation rate of entity x respectively; E_x is the population expansion coefficient of entity x after activated; $V_{IL6.Treg}$ is the maximum capability of modifying the differentiation towards Th17 by IL6 while TGF β is exist; $V_{IL1\beta.Treg}$ describes the maximum capability of suppressing the expansion of Treg cells. The fractions related to four cytokines represent the percentage of cells which the corresponding cytokines can induce from the primed Th0 cells. Since IL10 is a kind of immune suppression cytokines, it is considered as a suppression factor on expansion of Th1, Th2 and Treg cells.

After encountering the debris, resting microglia can be activated and differentiate into two types of effective microglia M1 and M2 according to its surrounding microenvironment. In this system, we choose IFN γ and IL4 as two important cytokines which can conduct the differentiation direction and we also consider the conversion between M1 and M2 based on the two cytokines. The ODEs for rM, M1 and M2 are expressed as

$$d[rM]/dt = P_{rM} - D_{rM} \cdot [rM] - C_{rM} \cdot H^1([Ag]) \cdot H^1([IFNr] + f \cdot [IL4]) \cdot [IM] \quad (6)$$

$$\begin{aligned} d[M1]/dt = & P_{M1} - D_{M1} \cdot [M1] \\ & + C_{rM} \cdot H^1([Ag]) \cdot H^1([IFNr] + f \cdot [IL4]) \cdot [IM] \cdot \frac{IFNr}{IFNr + f \cdot IL4} \cdot E_{M1} \\ & + C_{M2} \cdot H^2([IFNr]) \cdot [M2] \\ & - C_{M1} \cdot H^2([IL4]) \cdot [M1] \end{aligned} \quad (7)$$

$$\begin{aligned} d[M2]/dt = & P_{M2} - D_{M2} \cdot [M2] \\ & + C_{rM} \cdot H^1([Ag]) \cdot H^1([IFNr] + f \cdot [IL4]) \cdot [IM] \cdot \frac{f \cdot IL4}{IFNr + f \cdot IL4} \cdot E_{M2} \\ & + C_{M1} \cdot H^2([IL4]) \cdot [M1] \\ & - C_{M2} \cdot H^2([IFNr]) \cdot [M2] \end{aligned} \quad (8)$$

where C_{TM} is the conversion rate of rM; C_{MI} is the conversion rate from M1 to M2 conducted by IL4 and C_{M2} is the conversion rate from M2 to M1 conducted by IFN γ ; f is the relative weights which represent the capability of conducting differentiation direction by IL4 while compared to IFN γ . The fractions related with IFN γ and IL4 represent the percentage of cells which the corresponding cytokines can induce from Ag stimulated microglia.

In order to start the disease progression, we assume a constant conversion rate from nMN to mMN. As a result of steady population of motoneurons under physiological conditions and the incurability of ALS, we ignore the regular metabolism of motoneuron population for simplicity. The ODEs for describing the population dynamic of motoneurons are expressed as

$$d[nMN]/dt = -D_{MN} \cdot [nMN] \cdot H^2\left(\frac{[SO]}{[IGF1]}\right) - C_{nMN} \cdot [nMN] \quad (9)$$

$$d[mMN]/dt = -D_{MN} \cdot [mMN] \cdot H^2\left(\frac{[SO]}{[IGF1]}\right) + C_{nMN} \cdot [nMN] \quad (10)$$

where D_{MN} is the degradation rate of motoneurons and C_{nMN} is the conversion rate from nMN to mMN. The first terms on the right of Eqs. (9) and (10) represent the neurodegradation which is caused by SO and can be suppressed by IGF-1.

Ag is generated from neurodegradation of both nMN and mMN which can be expressed as

$$d[Ag]/dt = K_{DMN,Ag} \cdot D_{MN} \cdot ([nMN] + [mMN]) \cdot H^2\left(\frac{[SO]}{[IGF1]}\right) - D_{Ag} \cdot [Ag] \quad (11)$$

where $K_{DMN,Ag}$ is the generation rate of debris by neurodegradation and D_{Ag} is the degradation rate of debris.

In addition to mMN, mSOD1 is also assumed to be released by the necrosis of mMN. As a result, the population of mSOD1 is governed by

$$d[mSOD1]/dt = K_{mMN,mSOD1} \cdot [mMN] + K_{DMN,mSOD1} \cdot D_{MN} \cdot mMN \cdot H^2\left(\frac{[SO]}{[IGF1]}\right) - D_{mSOD1} \cdot [mSOD1] \quad (12)$$

where $K_{mMN,mSOD1}$ and $K_{DMN,mSOD1}$ are the release rate of mSOD1 by mMN and necrosis of mMN respectively; D_{mSOD1} is the degradation rate of mSOD1.

The ODEs describing the population dynamics of other cytokines and proteins generally expressed as

$$d[y_i]/dt = P_{y_i} - D_{y_i} \cdot [y_i] + \sum_{j=1}^N K_{Cellj,y_i} \cdot [Cell_j] \cdot \prod_{m=1}^M (1 \pm V_{y_m,y_i} \cdot H^1([y_m])) \quad (13)$$

where y_i is the i th cytokine or protein; P_{y_i} and D_{y_i} are the production rate and degradation rate of y_i respectively; K_{Cellj,y_i} is the release rate of y_i from the j th cell, which can be promoted (+) or inhibited (−) by y_m ; V_{y_m,y_i} represents the maximum effect of y_m on y_i .

Parameters Estimation

There are totally 20 ODEs and 70 parameters in this system model. For our simulations, we train these parameters to generate an optimized fitting to the time lapse data from [15]. In these experiments, mSOD1 transgenic murine models were used to investigate the effect of

T cells on modulating the disease progression. RNA was isolated from homogenized frozen spinal cord tissue of mSOD1 and age-matched wild-type mice at 11, 14, 16, 18 and 20 weeks of age, and at end stage (ES) disease. The relative expression level of several messenger RNA (mRNA) was obtained by employing the quantitative reverse transcriptase polymerase chain reaction technique (QRT-PCR). Although a single cell's protein and mRNA level for any given gene are uncorrelated, the protein level of a given gene is proportional to the mRNA level for a cluster of cells [18]. As a result, the relative mRNA level for each gene can be considered as the relative population of the entity with corresponding gene biomarker. The population of motor neurons is assumed to be correlated to the disease progress.

In order to produce an optimized fitting to the QRT-PCR data, we minimize the following objective function to obtain the estimate for all parameters

$$\theta = \arg \theta \in \Theta \min_j = 1/M_j = 1/N_{wi} \cdot |x_{iexp}(t_j) - x_{isim}(t_j)|^2, \quad \theta_2, w_i = 1/\max_j |x_{iexp}(t_j)|^2 \quad (14)$$

where θ is estimate of the parameters and Θ is the parameter space for θ ; $x_{iexp}(t_j)$ and $x_{isim}(t_j, \theta)$ represent the observed value of mRNA level from experiment and the calculated value of entity population from simulation with parameters θ at time point t_j , respectively. Genetic algorithm method is adopted as the search algorithm to minimize the objective function. In order to obtain a more reliable estimate for the parameters, we repeated the search algorithms many times with a large parameter space at first. Then we refine the parameter space according to estimate with low value for the objective function. Finally, after several repetition of this procedure, an optimized combination of parameters can be located in the parameter space to represent the mRNA level dynamics felicitously.

Results

Simulated Population Dynamics in Disease Progress without Interference

Fig. 2 shows the simulation results of population dynamics for each entity in this system trained by the experiment data. It can be seen that the simulation results is a good representation for the experiment data. Especially, the well fitted curve of $TGF\beta$ can be obtained only by adding the link from M1 microglia to $TGF\beta$. This indicates that M1 microglia may be another kind of cells which will release $TGF\beta$ in ALS disease progress. We can also see that Th2 cell is the only entity whose population changes a little throughout the disease progression, which may imply a little effect of Th2 cell involved in untreated disease progression. In spite of the dysfunction of mMN in producing SOD1, these cells still have the capability of processing and transmitting information by electrical and chemical signaling. As a result, the number of motor neurons (both nMN and mMN) is supposed to be correlated to the disease progression. Fig. 3 shows the total relative population of nMN and mMN in simulation and the murine disease progression data from [15]. It is indicated that the system model developed in this work can represent the switch of disease progression from an early stable phase into a later rapid phase. According to the results from the simulation, there are only 10.14% motor neurons survived at the end stage (113 day) of the disease. For subsequent analysis, we assume that if the number of motor neurons is lower than this percentage life cannot be supported and the disease turns into the end stage. Based on this assumption, the extended life is chosen as a final output of this system.

Sensitivity Analysis for Parameters

Parameter sensitivity analysis is a tool to examine whether the system is preserved to the modest parameter changes and quantitatively explore the sensitive parameters. In this work, local parameter sensitivity analysis is employed to study the relationship between survival

time and the variations for each parameter value. The sensitivity coefficient (S) is calculated according to the following formula:

$$S_i = \partial ST / \partial P_i / ST P_i \approx \Delta ST / \Delta P_i \quad (15)$$

where ST is the survival time; P_i is the i th estimated parameters in the system and ΔP_i is a small change of the corresponding parameter. In this work, each parameter is increased by 2% of its estimated value for investigating the corresponding change of survival time, as shown in Fig. 4. We can see that the survival time is sensitive to b , c , P_{T1} , D_{Treg} , $V_{IL1\beta.Treg}$, P_{Treg} , $D_{IFN\gamma}$ and $K_{T1.IFN\gamma}$ and the maximum change of survival time is about 1.8%. These parameters may reflect the essential difference among the patients with different survival time. This sensitive analysis (Fig. 4) also demonstrates that the system model developed in this work is rather robust.

Effect of different therapeutic strategies on ALS disease progress

Based on the system model parameterized as above, we can compare the predicted efficacy of several therapeutic approaches targeting the cells or proteins. In the prediction of each approach, therapeutic schedule and dose are considered as two major factors in conducting the treatment strategy to achieve an optimized effect.

Adaptive immune system is the upstream in modulating the disease progress, so we firstly investigate the effect of T cell based therapeutic strategies on ALS disease progress. For Th2 and Treg cells, which promote the neuroprotective response, therapies are carried out by injection of these cells while elimination of Th0 and Th1 cells can also exhibit a therapeutic effect. Fig. 5 shows the extended life after four therapeutic strategies targeting these four cells respectively. The injection or elimination are started from the 15th day when disease symptom is thought to emerge and repeated every 28 days, which is consistent with the in vivo T cell transference experiment from [15]. The simulation result indicates that elimination of Th1 cells can dramatically extend the survival time more effectively than other therapies. It can demonstrate the dominant effect of Th1 cells on triggering the later rapid development of disease progress. However, in clinic, specific elimination of Th1 cells is not always as feasible as specific injection of Treg or Th2 cells. Fig. 6 shows the population dynamic of Treg cells during the therapies with injecting different dosages of Treg cells. We can see that the injected Treg cells are observably suppressed at the end of each therapeutic cycle. The effect of this therapy on motor neurons is shown in Fig. 7. It is indicated that the stable phase of disease is extended and the disease symptom is alleviated throughout the therapy, which ultimately extends life span. As expected, the more dosage of Treg cells the mouse is received, the longer it will survive. However, high dosage injection is difficult to achieve, and according to the extended life - Treg dosage profile, the therapeutic efficiency will decrease when the dosage is larger than 3. As a result, additional clinical limitations and efficiency should be considered together to find out an optimized dosage for therapy.

In most cases, patients cannot receive the therapy as soon as the disease symptom emerges. The therapy start date will also alter the therapeutic effect on disease progression. Fig. 8 shows the effect of treatment started at different days with consistent dosage and therapeutic interval on motor neurons. It is indicated that the therapy which starts before stable phase of disease is more sensitive to the start date while compared to the later-starting treatment.

Cytokines are a category of signalling molecules used extensively in intercellular communication. They can also be treated as the target for disease therapy. Compared to cell transference, cytokines can be injected or eliminated more easily and effectively. As a result, we set the therapeutic interval to be 7 days for cytokine therapy. Fig. 9 shows the extended

life after several therapeutic strategies targeting cytokines and other entities respectively. We can see that elimination of Ag can remarkably extend life. Nevertheless, incorporating removal of debris of motor neurons in CNS into therapeutic strategies can be a challenging problem. As a result, we turn to investigate the other entities' effect on disease progress.

According to Fig. 9, IL6 can be considered as a potential therapeutic target to extend the life of patients with ALS. IL6 can up-regulate the synthesis of IL-4 by Th2 [19] and suppress the differentiation from Th0 into Treg cells [20]. However, direct injection of IL4, which shifts the balance of microglia responses from neurotoxicity to increased neuroprotection, cannot extend life longer than injection of IL6. Fig. 10 shows the effect of IL6 injection on IL4, Treg cells, M1 microglia and M2 microglia compared with therapy targeting IL4. We can see that although the population of IL4 can achieve a high peak by injecting IL4 directly, it decays rapidly in each therapeutic cycle and results in limited benefit on neuroprotective response. In contrast, injected IL6 can prevent the decay of IL4 and result in elevation of the IL4 level at the later stage of the disease. Finally, the neuroprotective response caused by elevated ratio of M2 microglia to M1 microglia at the later stage will effectively prevent the degradation of motor neurons to extend life. Fig. 11(a) shows the extended life after injection of IL6 or IL4 with different dosages. It can be seen that the extended days by IL6 therapy increases about 4 fold faster than IL4 therapy when the dosage is lower than 4. If the dosage continues to increase, efficiency of IL6 therapy will decrease to the same level as IL4 therapy. This dosage can be used as reference for optimizing the IL6 therapy when side effects are considered. Fig. 11(b) shows therapeutic effect of constant dosage injection of IL6 or IL4 started from different days. It indicates that therapy started before the rapid phase of disease progression will significantly extend the survival time while the later therapy is started in the rapid phase, the less effect it provides.

IFN γ is the inflammatory cytokine which can promote the development of ALS disease. Corresponding inhibitors can be injected to neutralize these cytokines to slow down the disease progression and then extend the survival duration. Fig. 12 shows the population dynamics of IFN γ targeted by therapy. It is indicated that after elimination, it takes about 3 days for IFN γ to recover. The therapeutic effect on motor neurons is shown in Fig. 13. We can see that, due to the low population of IFN γ , this therapy has less influence on disease progression during the stable phase. However, later elimination of IFN γ can maintain the stable phase for an additional period. Similar to IL4, the therapeutic effect of IFN γ increases with elimination percentage linearly. Fig. 14 shows the relative population of motor neurons with the elimination of 40% IFN γ performed from different start dates.

Conclusions

In this work, we have established a mathematical model of cell-cell communication network in ALS disease using ODEs. In addition to the cells, our model also integrates a variety of soluble signaling proteins, e.g. cytokines, which are the key components mediating the intercellular communication in the microenvironment. After the refinement of parameters using experiment data, this model provides us some insights into the mechanism at the system level how these cells and their communication collectively modulate the disease progression.

According to the experiment data, TGF β , an anti-inflammatory cytokine, continued to increase at the later stage of disease progression while the Treg cells and M2 microglia which are considered as the major sources of such cytokine in this system decreased and cannot supply enough TGF β . In order to perform an optimized fit to the experiment data, we tried to add several links from different cells to TGF β . Finally, M1 microglia was figured out as another minor source of TGF β [21]. This may imply that after uptake of apoptotic

motor neurons, M1 microglia tries to exert some anti-inflammatory effects by increased expression of TGF β [22]. However, this effect is limited and difficult to turn over the rapid progressive disorder of ALS.

Sensitivity analysis of this model helped us characterize parameters with significant impacts on the system. We did a local sensitivity analysis by increase each parameter by 2% and study their effects on output of the model. In this study, we choose the survival time, which is based on the population of motor neurons, as the model output. We found that P_{Th1} , $K_{Th1,IFN\gamma}$, $D_{IFN\gamma}$ and $V_{IL1\beta, Treg}$ are the most sensitive parameters. This implies that, either slowing the accumulation of IFN γ released by Th1 cells or decreasing the suppression effect of IL1 β on Treg cell expansion will retard the development of disease. These factors may be related to the inter-patient heterogeneity for the reason why some people with ALS can survive for a long time while others die soon. Furthermore, this also provides several potential intracellular targets related to these parameters, e.g. IFN γ synthesis pathway in Th1 cell.

Several designed therapeutic strategies targeting the cells or cytokines were tested in this system. Among the T cell based therapies, elimination of Th1 cells seems to be the most effective approach that could retard the disease progression. This also confirms the dominant role of Th1 cells in shifting the microglia response from neuroprotective into neurotoxic. The test on injection of Treg cells suggests that this therapy has the capability of preventing the degradation of motor neurons in a dose-dependent manner and the start date of therapy is closely related to the survival time, especially in the rapid phase of disease. For those therapies targeting cytokines, injection of IL6 can essentially elevate the level of IL4, which can augment the neuroprotective response, while direct injected IL4 will decay rapidly in such a microenvironment. This is due to the persistent stimulation of IL6 on Th2 cells to release IL4. On the other hand, elimination of mSOD1 or Ag is a direct approach to suppress the activation of microglia and indeed extends the survival time effectively in our simulation. However, direct elimination of mSOD1 or Ag in CNS is difficult. As an alternative, elimination of IFN γ , which plays a role in inflammation-induced motoneuron death [23], can be considered as an effective therapy to extend the lifespan of patients with ALS.

Taken together, this mathematical model can provide a better understand of intercellular communication in ALS disease progression at the system level. Furthermore, if we combine the side effect of different drugs, it can be used to seek the potential targets and optimize the therapeutic strategies so that they can effectively improve survival rates and quality of life for patients with ALS.

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Fig. 1. The schematic diagram for primary interactions between immune system and CNS in mSOD1-induced ALS. rM: resting microglia; SO: Superoxide.

Fig. 2. Population dynamics of each entity from simulation results compared with experiment data.

Fig. 3. Simulated relative population of motor neurons compared with the disease progress.

Fig. 4.
Sensitivity of survival time to the increase of each parameter

Fig. 5.
Extended life after four therapeutic strategies targeting adaptive immune cells

Fig. 6.
Population dynamics of Treg cells during the therapies using different dosages.

Fig. 7.
Relative population of motor neurons during the Treg cell based therapy with different dosages.

Fig. 8.
Relative population of motor neurons during the Treg cell based therapy with different start dates.

Fig. 9.
Extended life after several therapeutic strategies targeting cytokines and proteins.

Fig. 10.
Effect of IL6 injection on IL4, Treg cells, M1 microglia and M2 microglia compared with IL4 injection.

Fig. 11.
Effect of IL6 or IL4 injection on extended survival time.
a) Different dosages. b) Different start days.

Fig. 12.
Population dynamics of IFN γ during the IFN γ -eliminating therapy..

Fig. 13. Population dynamics of motor neurons during the IFN γ -eliminating therapy with different dosages.

Fig. 14. Population dynamics of motor neurons during the IFN γ -eliminating therapy with different start dates.