



ISTITUTO DI ANALISI DEI SISTEMI ED INFORMATICA
"Antonio Ruberti"

CONSIGLIO NAZIONALE DELLE RICERCHE

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MODELING REJECTION IMMUNITY

R. 10-10 2010

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ISSN: 1128–3378

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Abstract

Transplantation is often the outcome of a number of diseases leading to organ failure. To overcome rejection towards the transplanted organ (graft), immunosuppression therapies are used, which have considerable side-effects and expose patients to opportunistic infections. The development of a model to complement the physicians experience in specifying therapeutic regimens is therefore desirable. The present work proposes an Ordinary Differential Equations model accounting for immune cells proliferation in response to the sudden entry of graft antigens, through different activation mechanisms. The model considers the effect of a single immunosuppressive medication (e.g. cyclosporine), subject to first-order linear kinetics and acting by modifying, in a saturable concentration-dependent fashion, the proliferation coefficient. The model proposed substantially simplifies the chain of events potentially leading to organ rejection. It is however able to simulate quantitatively the time course of graft-related antigen and competent immunoreactive cell populations, showing the long-term alternative outcomes of rejection, tolerance or tolerance at a reduced functional tissue mass. In particular, the model shows that it may be difficult to attain tolerance at full tissue mass with acceptably low doses of a single immunosuppressant, in accord with clinical experience. The model has been formalized so as to be directly extended to incorporate several immunosuppressive agents, acting at different levels of the mechanism leading to rejection, and to accommodate additional specific cell populations or specific antigen types as needed. This formalization lends itself specifically to the simulation of different therapy schemes, supporting the optimal clinical use of available agents.

Key words: organ transplantation, ODE, T-lymphocytes dynamics, immune reaction, rejection therapy

Modeling Rejection Immunity

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July 29, 2010

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1 Abstract

Transplantation is often the outcome of a number of diseases leading to organ failure. To overcome rejection towards the transplanted organ (graft), immunosuppression therapies are used, which have considerable side-effects and expose patients to opportunistic infections. The development of a model to complement the physician's experience in specifying therapeutic regimens is therefore desirable.

The present work proposes an Ordinary Differential Equations model accounting for immune cells proliferation in response to the sudden entry of graft antigens, through different activation mechanisms. The model considers the effect of a single immunosuppressive medication (*e.g.* cyclosporine), subject to first-order linear kinetics and acting by modifying, in a saturable concentration-dependent fashion, the proliferation coefficient.

The model proposed substantially simplifies the chain of events potentially leading to organ rejection. It is however able to simulate quantitatively the time course of graft-related antigen and competent immunoreactive cell populations, showing the long-term alternative outcomes of rejection, tolerance or tolerance at a reduced functional tissue mass. In particular, the model shows that it may be difficult to attain tolerance at full tissue mass with acceptably low doses of a single immunosuppressant, in accord with clinical experience.

The model has been formalized so as to be directly extended to incorporate several immunosuppressive agents, acting at different levels of the mechanism leading to rejection, and to accommodate additional specific cell populations or specific antigen types as needed. This formalization lends itself specifically to the simulation of different therapy schemes, supporting the optimal clinical use of available agents.

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2 Introduction

It is unfortunately not rare in medical practice that some diseases lead to organ failure, which may eventually require organ transplantation. The liver, the kidney and the heart are the most frequently transplanted organs. Diseases leading to organ transplantation span a wide spectrum of medical conditions: cancer, infections, autoimmune degenerative disease. Transplantation into the recipient of a foreign organ (graft), even from an individual of the same species (allograft), if left to itself causes rejection, a strong response by the recipient's immune system leading to irreversible damage of the graft. Depending on the time-frame over which rejection occurs, clinicians use to differentiate "acute" from "chronic" rejection. Acute rejection develops in the first few weeks or months after transplantation and is produced by cellular and molecular mechanisms, which may partially differ from those leading, over the course of many months or years, to chronic rejection.

After many decades of experimentation on animals and cells, and of development of pharmacological tools, organ transplantation has evolved into a common therapeutic procedure. The success of a solid organ transplant relies in equal measure on the technical aspects of the implant and on the recipient's acceptance or tolerance of the implanted graft. This last phenomenon is clinically induced by the administration of immunosuppressive drugs, which specifically decrease the recipient's reactivity towards the graft, thus allowing the maintenance of the functional activity of the organ. The currently available drugs belong to several classes (calcineurine inhibitors (CNI), antimetabolites, target of rapamycin (TOR) inhibitors, steroids, monoclonal antibodies) (Taylor et al., 2005). Canonical combinations of these drugs are typically used by the attending physician in a rather standardized fashion, attempting to maintain measurable drug plasma concentrations within established limits. Episodic use of emergency therapy is then performed if and when signs of rejection become clinically evident.

The acute rejection response has been extensively studied *in vivo* and *in vitro*. From the available studies, indications on the action of several drugs in controlling acute rejection and maintaining vitality of the graft have been obtained. However, even if the recipient initially accepts the graft, the more insidious and slow phenomenon of chronic rejection often ensues. The pathogenesis of chronic rejection is less well known and may depend not only on a partially different set of immune response mechanisms, but also on the ac-

tual drug toxicity, recommending the use of the minimal clinically effective dose of medication.

Rejection has been widely described from a medical and biological viewpoint, but there have been so far no mathematical models describing this process. Mathematical models have been used to describe immunological behavior for a long time, beginning from the classical SIR model (Susceptible-Infectious-Recovered) first created for investigating the progress of an epidemic (Kermack and McKendrick, 1927). Several models describing the immune response in a number of diseases exist (HIV infection, tuberculosis, tumors...) (Bajaria et al., 2002; Marino and Krischner 2004; Matzavinos et al., 2004; Flower and Timmis, 2007) but, at present, only one model representing immune system dynamics during transplantation (Ciupe et al., 2009) has been published, which has the important aim to investigate T-cell population growth mechanisms, using thymus transplantation to follow the development of T-cells and their regulatory signals. The goal of the present work is somewhat different, in that we attempt to describe relevant features of the immune system dynamics during general solid organ rejection. This will allow in the future the description of possible consequences of different therapeutic regimens. From the immunologic viewpoint, rejection mechanisms are substantially different from other immune responses (*e.g.* from HIV, tuberculosis, or tumors), and the development of a specific model seems therefore warranted. Such a model should help transplantation clinicians and allied health care personnel in forecasting and treating rejection, without relying solely on empirical protocols. A good mathematical model should eventually allow the physician to keep into simultaneous consideration the several interrelated aspects of the immune response to transplantation, besides incorporating the known pharmacokinetics of the many potentially useful available drugs. The ultimate goal would be to help bridge the gap between the pharmacology and the biology of transplantation, explaining or at least representing the temporal relations between drug efficacy, possible drug adverse effects and the development of immune tolerance or graft acceptance.

In the present work we propose a tentative mathematical model of the rejection towards a solid organ transplant (kidney, liver, pancreas, heart). This model describes the evolution of the main cellular immune response as well as the kinetics and action of a single representative drug (cyclosporine). While the presented model is rather basic, it will introduce the main elements needed for the eventual description of more detailed response dynam-

ics. Three case scenarios are discussed: non-immunosuppressed transplantation and two immunosuppressive therapeutical regimens, one with moderate drug dose and the other with high drug dose.

3 Materials and Methods

3.1 Relevant Physiology

During the early development of an individual, the immune system learns to recognize "self" molecules and not to react to them: "non-self" and "self" indicate whether a molecule is foreign or not, regardless of its coming from outside the body. The prefix "allo" indicates different organism of the same species (allograft, alloantigen). After transplantation, the majority of exogenous molecules on the allograft are recognized by the immune system as self antigens (because they are the same in both donor and recipient, *e.g.* collagen or haemoglobin), while only few donor molecules, mostly proteins, actually induce the immune response. Many of these proteins are coded by genes belonging to a locus known as the Major Histocompatibility Complex (MHC). Their role is mainly to bind epitopes (active portions of larger antigen molecules, which stimulate the immune response), and exhibit them on the surface of Antigen Presenting Cells (APCs). T Cell Receptors (TCRs) on the surface of T-lymphocytes can recognize and bind the MHC/epitope complexes, thereby inducing the (cellular) immune response. MHC genes are the most polymorphic of the human genome, and there is essentially no possibility of finding two persons with the same gene cluster (excluding, of course, monozygotic twins) (Forsdyke, 1991).

MHCs already present on the donor cells (particularly on donor APCs) are the major targets of the immune response towards a transplanted organ (Cote et al., 2001). APCs are in fact normally produced from lymphoid organs, circulate, and are found in every organ in the donor: with transplantation many donor APCs, carrying donor MHCs binding various peptides, will thus be introduced in the body of the recipient, ready to stimulate the immune response.

In the conventional immune response to external antigens (*e.g.* from invading bacteria), foreign epitopes are bound to self-MHC molecules, which then prime T-cells for clonal expansion and useful immune response. On the other hand, an allograft carries its own set of MHC molecules, which are also effective in priming recipient T-cells, since recipient T-cell clones can recognize complexes formed by allogenic MHCs (Heeger, 2003). Our immune system

can therefore be activated directly or indirectly by the allograft, because the TCRs can be activated either by non self-MHC/epitope complexes ("direct activation", since it exploits ready-made and exposed MHC/epitope complexes), or by self-MHC/epitope complexes ("indirect activation", since it requires processing of the antigen to expose appropriately the corresponding MHC-epitope complex) (Gould and Auchincloss, 1999). It should be noticed that in the indirect mechanism, many of the foreign epitopes involved are in fact donor MHC fragments, due to the polymorphism mentioned above.

Every different MHC on the surface of an APC can be recognized by and activate to proliferation (clonal expansion) a different T-cell clone. When the direct mechanism is involved, the activation is faster, because alloantigens do not need to be captured and processed by APCs, once they are normally present on the surface of donor APCs (they do not evoke a response in the donor, being recognized there as "self"). On the other hand, when the indirect pathway is involved, alloantigens must first be captured and processed by the recipients' APCs and then exposed as non-self epitopes: this mechanism of activation is therefore slower.

Many of the antigenic peptides that are presented to recipient T lymphocytes through the indirect pathway derive from the polymorphic regions of the donor MHC molecules (Benichou et al., 1992). However, indirect activation can also occur in response to peptides derived from other molecules present in the allograft. "Alloreactive T lymphocytes are [*in any case*] requisite mediators of allograft rejection" (Heeger, 2003).

There are two types of T cells involved in rejection, CD4+ and CD8+ T-lymphocytes, which have different functions (respectively "helper" and "cytotoxic"): since both increase in numbers during the response to the allograft, at the level of detail used in the present work for modeling rejection, we only consider the total number of T cells. This choice is also supported by the fact that CD4+ and CD8+ can both be activated by exogenous antigens. There are in fact two different types of MHC molecules, named class I and class II, codified by two different loci. Exogenous antigen, endocytosed by APCs, is usually presented through the class II MHC antigen-processing pathway, activating graft-reactive CD4+ T cells. The class I MHC processing pathway was previously thought to exclusively present intracellular antigens. It is now clear, however, that class I MHC processing also permits presentation of extracellular antigen (Yewdell and Bennink, 1999) and activation of CD8+ T lymphocytes (this phenomenon is called cross-priming).

Direct T-cell activation is a time-limited process. In fact, in the course of direct activation, donor APCs are destroyed (Heeger, 2003). Since donor APCs do not reproduce in the transplanted organ, T-cells respond through the direct mechanism only in the early stage of rejection. However, the immune response to transplantation does not terminate when all exogenous APCs die: there is in fact a continuous supply of donor-specific molecules, produced from the constantly proliferating cells in the graft, which maintain immune activation. T-lymphocyte indirect activation occurs for as long as the graft is present in the recipient, since recipient's APCs migrating to the organ constantly process donor antigens.

We can thus appreciate two main phases of the immune response to the graft: an initial strong response, sustained by the direct activation of T-cells due to a large but fading quantity of donor APCs, necessitating aggressive immune suppression therapy; and a later, more or less constant indirect activation of T-cells, sustained by continuously produced graft epitopes, for which less aggressive therapy is sufficient. It has in fact been reported that the number of directly activated T-cells is larger than the number of indirectly-activated T-cells, the latter constituting less than 10 percent of the total cellular alloimmune repertoire (Benichou et al., 1999).

Two aspects of graft rejection have not been explicitly included in the model for simplicity. The first is the increased production of lymphocytes from lymphoid organs, which receive various signals from stimulating molecules (cytokines). This mechanism has been shown (Faderl and Estrov, 2000) to take place when an inflammatory process (*i.e.* rejection) is ongoing. The second aspect of rejection is the appearance of the graft-*versus*-host disease, where donor's T-cells present in the allograft react towards recipient's antigens. This last phenomenon depends on the type of the transplanted organ and may be negligible in most cases of solid organ transplantation.

In the present model we consider one of the most frequently used immunosuppression therapy protocols, based on calcineurine inhibitors (*e.g.* cyclosporine)(Taylor et al., 2005), which block T-cell clonal expansion. Drugs of this class inhibit signal transduction when TCRs recognize the epitope, so that the cell does not proliferate even when activated. Other types of therapy, acting through different mechanisms, could also be considered, extending the present approach.

3.2 The Model

The model has been represented in a block diagram (figure 1) showing all compartments and the relative dynamics. For clarity a single T-Lymphocyte compartment is shown, while in the model there are different compartments, each corresponding to a different antigen type.

Organ transplantation is assumed to occur at time t_τ in the life of the patient. Before transplantation, the major components of the immune system involved in transplant rejection are assumed to be at equilibrium. The choice of the time t_0 , at which simulations begin, is therefore irrelevant, as long as $t_0 < t_\tau$. For ease of consideration of the dynamics following transplantation, we use time units of days, even though the sample simulations shown below are reported with time in years for clarity. In order to represent organ damage, we assume that antigen is released into the blood stream proportionally with the viable mass of the corresponding tissue, and consequently that the viable graft mass is proportional to bloodstream antigen mass, so that a substantial decrease in antigen concentration will indicate organ failure.

With the transplantation of an allograft at time $t = t_\tau$, the state of the immune system is suddenly and dramatically altered. The entry of a large amount of already processed foreign antigen, and the continuous production of foreign antigen by the functional graft tissues, induce a strong response by the host's immune system. If this response is not controlled by immunosuppression, rejection and loss of the organ follow. Depending on the time and mechanisms needed to activate the immune response and on the duration of antigen permanence in the body, we describe different antigen types and assume that a specific T-cell type corresponds to each antigen type. In our model we use differential equations to describe antigen and T-cell dynamics, using appropriate matrices of coefficients to specify the effect of antigens on corresponding T-cells and viceversa.

The three main model equations describe the major variables implicated in this mechanism:

$$\frac{d\mathbf{a}(t)}{dt} = \mathbf{g} - \mathbf{K}_{XAC}\mathbf{C}\mathbf{a} - \mathbf{K}_{XAA}\mathbf{a} + \mathbf{Q}\mathbf{a}, \quad \mathbf{a}(0) = \mathbf{a}_0 \quad (1)$$

$$\frac{d\mathbf{c}(t)}{dt} = \mathbf{k}_C + \mathbf{K}_{CAC}(F)\mathbf{A}\mathbf{c} - \mathbf{K}_{XCA}\mathbf{A}\mathbf{c} - k_{xc}\mathbf{c}, \quad \mathbf{c}(0) = \mathbf{c}_0 \quad (2)$$

$$\frac{dF(t)}{dt} = k_f - k_{xf}F + \frac{\kappa_f}{k_{xf}}\delta(t - t_\tau), \quad F(0) = 0 \quad (3)$$

In equation 1, \mathbf{a} represents the amounts of five typical classes of antigens, which obey mass balance with: external inputs; elimination through the interaction with different classes of specifically primed lymphocytes (appearing as the elements of the vector \mathbf{c} or the diagonal matrix \mathbf{C}); elimination by unspecific mass effect; transfer from one to the other antigen form.

In equation 2 the amounts of the different types of lymphocytes evolve according to: some constant production; some proliferation, depending on the current drug concentration F , induced by the corresponding antigens (as elements of the diagonal matrix \mathbf{A}); elimination depending on antigen amounts; antigen-independent mortality (supposed to be the same for all types of lymphocytes).

Equation 3 represents the pharmacokinetics of the anti-rejection drug F with: given constant entry (depending on the administration scheme); linear elimination; impulsive entry assumed to be simultaneous with transplantation. The equations below and tables 1 and 2 define each symbol used.

$$\mathbf{a} = \left(E, L, U, Y, Z \right)' \quad (4)$$

$$\mathbf{g} = \mathbf{g}_1 + \mathbf{g}_2 \quad (5)$$

$$\mathbf{g}_1 = \left(k_e, L_{reg}, 0, 0, 0 \right)' \quad (6)$$

$$L_{reg} = L_{regmax} \frac{L}{k_{1/2} + L} \quad (7)$$

$$\mathbf{g}_2 = \left(0, k_{l\tau}, k_{u\tau}, k_{y\tau}, 0 \right)' \quad (8)$$

$$k_{l\tau} = \delta(t - t_\tau)L_\tau \quad (9)$$

$$k_{u\tau} = \delta(t - t_\tau)U_\tau \quad (10)$$

$$k_{y\tau} = \delta(t - t_\tau)Y_\tau \quad (11)$$

$$\mathbf{K}_{XAC} = \text{diag} \left(k_{xec}, k_{xlc}, k_{xuc}, 0, k_{xzc} \right)' \quad (12)$$

$$\mathbf{K}_{XA} = \text{diag} \left(k_{xe}, k_{xl}, k_{xu}, k_{xy}, k_{xz} \right)' \quad (13)$$

$$\mathbf{A} = \text{diag} \left(\mathbf{a} \right) \quad (14)$$

$$\mathbf{c} = \left(C_e, C_l, C_u, 0, C_z \right)' \quad (15)$$

$$\mathbf{C} = \text{diag} \left(\mathbf{c} \right) \quad (16)$$

$$\mathbf{Q} = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -k_{zy} & 0 \\ 0 & 0 & 0 & k_{zy} & 0 \end{pmatrix} \quad (17)$$

$$\mathbf{k}_C = \left(k_c, k_c, k_c, 0, k_c \right)' \quad (18)$$

$$\mathbf{K}_{CAC} = \text{diag} \left(K_{cac(F)}, K_{cac(F)}, K_{cac(F)}, 0, K_{cac(F)} \right)' \quad (19)$$

$$\mathbf{K}_{XCA} = \text{diag} \left(k_{xca}, k_{xca}, k_{xca}, 0, k_{xca} \right)' \quad (20)$$

$$k_f = \begin{cases} 0, & \text{if } t \leq t_\tau \\ \kappa_f, & \text{if } t > t_\tau \end{cases} \quad (21)$$

$$K_{cac}(F) = k_{cac} F e^{-\lambda F} \quad (22)$$

In equation 1, \mathbf{a} is a five elements vector (equation 4), where every element is the quantity of different antigen type in the body. E is a generic environmental antigen: we suppose that the individual is constantly exposed to bacteria, viruses etc. and that the immune system is continuously stimulated by the corresponding exogenous antigens. When the allograft is transplanted in the recipient's body, however, different antigen types are introduced. L represents antigen produced by graft cells: while these cells reproduce, this antigen is continuously formed. U indicates alloantigen directly presented by donor's APCs, which does not form anew in the organ. This antigen is ready to be presented to T-cells, so its effect in activating T-lymphocytes is rapid and strong, but vanishes as APCs are progressively cleared from the organ. Alloantigen indirectly presented and deriving from cells not reproducing in the allograft, necessitates processing by endogenous APCs and presentation to T-cells. How was explained in the previous section, this antigen does not remain in the recipient once it is eliminated. Y represents the unprocessed form, while Z represents the antigen once processed and ready to activate T-cells. The distinction between the processed and the unprocessed form is used to create a delay in this kind of antigen effect in respect of the directly presented ones.

In equation 1, describing antigen dynamics, \mathbf{g} is the vector representing the entry rates of the several types of antigen. It is given in equation 5 as the sum of two components, g_1 and g_2 . In the sum of two vectors: vector g_1 collects for each antigen the continuous rates of entry into the system. These are zero except for E and L. For E, the rate k_e simply represents the entry

of environmental antigens into the body, assumed to be constant throughout the considered period of time (before as well as after transplantation). L_{reg} describes the continuous production of antigen L, the antigen associated with regenerating graft tissues. Equation 7 defines this production rate as depending on the current antigen mass L, on the maximal regeneration rate L_{regmax} and on a rate parameter $k_{1/2}$, following a standard nonlinear, saturating relationship.

Vector g_2 (equation 8) collects for each antigen the impulsive entries into the system. These are zero except for L, U and Y. The three non zero elements of g_2 are defined individually in equations 9, 10 and 11. In each case an antigen concentration (respectively L_τ , U_τ and Y_τ) is multiplied by a Dirac delta term entered at t_τ . The end result is the representation of the appropriate jump change in the L, U and Y compartments at the time of transplantation. The elements of both g_1 and g_2 corresponding to Z are 0 because the processed form of the antigen derives from the unprocessed form Y (see later) and has no direct entry, either continuous or impulsive.

The elimination terms $K_{XAC}\mathbf{Ca}$ and $K_{XAA}\mathbf{a}$ describe antigen neutralization due to respectively T-cell action and T-cell-independent elimination of the antigen (as it happens *e.g.* through chemical and physical elimination mechanisms, such as lipases, mucus secreted by respiratory and gastrointestinal tracts etc.).

The term \mathbf{Qa} is used to indicate the transfer rate from the unprocessed form (Y) to the processed form (Z) of the indirectly presented antigen. In fact, all elements of matrix \mathbf{Q} (17) are 0, with the exception of those multiplying Y and Z: for Y the transfer rate is a negative one ($-k_{zy}$), because the antigen is processed and goes from compartment Y to Z. For Z, k_{zy} is positive and it actually represents the only entry into the compartment, since, as seen before, the element in \mathbf{g} corresponding to Z is 0.

Equation 2 describes T-cells dynamics, vector \mathbf{c} (15) representing antigen-specific lymphocytes. We assume that antigen action on T-cell proliferation and death reflects the same mechanisms, both for environmental and graft antigens. \mathbf{K}_C (18) indicates constant physiological T-lymphocyte production from lymphoid organs, $\mathbf{K}_{CAC(F)}\mathbf{Ac}$ (19) represents T-cell clonal expansion after antigen contact, which is inhibited by drug action. T-lymphocytes are also "consumed" by antigen, in the sense that upon T-cell interaction with antigen the lymphocyte eventually undergoes apoptosis (programmed cell death): this is described by the elimination term $\mathbf{K}_{XCA}\mathbf{Ac}$ (20). We

introduced another elimination term, $k_{xc}\mathbf{c}$: lymphocytes die for apoptosis if they do not encounter any antigen after a certain period, and we assume this mechanism to be proportional to T-cell concentration. Notice that in the T-cell equation every element corresponding to unprocessed antigen is 0, in fact, this form is not ready to activate T-lymphocytes.

Cyclosporine pharmacokinetics is described by equation 3. For the purpose of the present model, given the long time-scale considered, drug administration is assumed to be continuous, with average rate k_f (equation 21) of delivery into the circulation. The drug is eliminated from the circulation following a linear, first-order process with rate constant k_{xf} . As before transplantation no treatment is employed, k_f is 0 before time t_τ , while it is equal to κ_f from the time at which therapy begins, which we assume to be t_τ . The term $\frac{\kappa_f}{k_{xf}}\delta(t-t_\tau)$ represents the (impulsive) loading dose of the drug, necessary to bring it rapidly to the desired level, at which it is then assumed to be constant. After antigen contact, T-cells (C) proliferate (clonal expansion). Equation 22 describes the cyclosporine (F) inhibitory effect through the function $K_{cac(F)}$: the drug blocks signal transduction after antigen contact, in a concentration-dependent fashion, thus disabling clonal expansion and reducing T-cell proliferation.

4 Results

The model has been implemented in Matlab©2006b, and simulations are presented showing the behavior of the several types of antigen and corresponding specific T-cell populations after transplantation of a solid organ. Three scenarios are depicted, corresponding respectively to the no-therapy, moderate therapy and maximal therapy situations.

On the x-axis, time in years is indicated: we choose a time range from 35 to 60 years, hypothesizing that transplantation occurs at time 40 years. In figure 2 all antigen types, the corresponding T-cell dynamics, and drug concentrations, are shown in the three therapy cases. In all subfigures solid line (—) represents the no-therapy case, the dashed line (- -) refers to the moderate drug dose while the dotted line (..) refers to the high drug dose.

Subfigures 2.10, 2.11 and 2.12 show respectively drug dynamics, total antigen dynamics (as the sum of specific antigens) and total T-lymphocytes dynamics (as the sum of antigen specific lymphocytes).

Concentrations are assumed to be constant if no traumatic events happen during life. As the allograft is introduced ($t_\tau = 40$ years), all antigen types described by the model (with the exception of the environmental antigen E) go from 0 to a high level, and the total antigen suddenly increases. In response, total T-cell concentration also grows from a normal, low level to a high one. The solid line corresponds to the no-therapy case: at the beginning antigen concentration is very high and so T-cells are activated causing antigen elimination, which only partially regenerates (case of antigen L). After this first period, as T-cells react towards the graft to destroy it, antigen concentration (which is proportional to graft mass) decreases. Following antigen reduction, T-cell concentration is also reduced, but remains consistently higher than physiological (*i.e.* before transplantation) concentration, as antigen from the organ is continuously produced and never vanishes. If no therapy is applied, T-cell levels remain high and will bring the organ to rejection and failure: as can be noticed, the continuous line in the total antigen graph is very low.

The dashed line represents the case in which administration of a moderate amount of drug (cyclosporine in this case) takes place. We assume that the drug is given simultaneously with the organ transplantation and that the patient is continuously and constantly treated (subfigure 2.10). It should be noticed that, as the increase of T-cell concentration is much lower, antigen level remains higher than the no-therapy case. This indicates that the graft is not totally destroyed by the immune system.

If therapy is much stronger (dotted line), the graft normally survives. The problem is that, with immunosuppression, T-cell levels fall well below normal. In fact, while rejection is prevented, the patients might not be able to defend themselves from severe general infections. As T-cells are less aggressively attacking the allograft, its regeneration allows the attainment of a constant equilibrium level, but T-cells do not proliferate as much and are thereby less effective not only towards the graft's antigen, but also towards environmental antigens, exposing the patient to opportunistic infections. In fact, any chosen intensity of therapy represents a compromise between desirable graft tolerance and dangerous lowering of general immune defenses.

Subfigures 2.1 to 2.9 show the time course of specific antigens and their respective T-cell dynamics. In subfigures 2.1 and 2.2, generic antigen and generic T-lymphocyte concentrations are reported. If no therapy is admin-

istered, these dynamics are at equilibrium. With therapy, generic T-cell concentration decreases (depending on drug dose): this indicates that immunosuppression it is not specific in lowering T-cell expansion towards the graft, instead, it reduces proliferation of all T-cells and, as a consequence, environmental antigen (E) increases, albeit so modestly that this increase cannot be readily appreciated in the reported graph.

In subfigures 2.3 and 2.4, the concentration level of regenerating antigen (L) and T-cells which respond to it (C_L), is shown. As seen before, in the absence of therapy, low antigen and high T-cell levels are reached after transplantation, while with drug administration antigen and T-cell concentrations are respectively higher and lower. This is the only case in which antigen never goes to zero because it is produced from regenerating graft cells. Dynamics of directly presented (U) and indirectly presented (Z) antigen (subfigures 2.5 and 2.8) are similar, with the difference that, as the indirectly presented antigen has to be processed, there is a delay in its increase, which depends on non-processed antigen (Y) dynamics. This is shown in graph 2.7: the unprocessed antigen rapidly grows and rapidly vanishes as it is processed to the indirectly presented form and is no longer produced (because it derives from APCs not reproducing in the graft). There are no specific T-cells towards this antigen type as the non-processed form cannot activate them.

In subfigures 2.6 and 2.9 the dynamics of specific T-cells primed for antigen U and Z dynamics are shown. In these cases as well, as drug dosages increase, T-cell concentrations decrease and antigen levels rise.

From the graphs it is evident that is not easy to find the right drug dose, in fact it can be seen that when the dose is moderate T-cell level is adequate but the antigen, and thereby the graft tissue, are still too low, while, as drug concentration increases, T-lymphocyte level is not sufficient to defend the patient from infections. The perfect situation will be to find the therapy level which is effective in saving the graft from inflammation but does not expose the individual to diseases.

5 Discussion

Mathematical models are increasingly being used in biology and clinical medicine to express concise, mechanistic descriptions of ongoing phenomena. The possibility of representing a pathophysiological process by means

of a mathematical model allows the investigator to formalize beliefs, compare interpretations and simulate hypothetical scenarios of interest. Mathematical models have in particular already been introduced in several areas of immunology (Bajaria et al., 2002; Marino and Krischner 2004; Matzavinos et al.: 2004; Flower and Timmis, 2007; Ciupe et al., 2009). So far, however, no mathematical model has yet been presented describing rejection in order to ease therapy development.

The clinical problem, which characterizes the management of the transplanted patient, is the difficult adjustment of immunosuppressive therapy, walking the fine line between under-suppression, with ensuing organ rejection, and over-suppression, with the danger of potentially lethal opportunistic infections. While a wide spectrum of active pharmacological agents are now available to the transplantation specialist, their mechanism of action is often incompletely known and their precise effect on the complex balance of immune system competence is not quantitatively determined. Therapy therefore follows rule-of-thumb principles, intensive monitoring of potential damage indicators (like serum creatinine for kidney, hepatic enzymes for liver transplantation, meticulous monitoring of plasma drug levels). The relationship between the time courses of drug effect, T-cell cycle and organ damage is however a matter of guesswork, only partially mitigated by the relatively precise knowledge of the pharmacokinetics of the immunosuppressive drugs themselves. In fact, what pharmacological information is currently offered to clinicians consists largely of single-drug pharmacokinetic parameters.

In this context, the formal representation and computer implementation of (a simplified version of) commonly accepted mechanisms allows first the reproduction, by way of simulation, of idealized versions of actually observed clinical phenomena, then the investigation of hypothetical scenarios, testing (*in silico*) the plausibility and completeness of the simplifying assumptions made. Crucial in this respect is the reproduction of expected qualitative behavior by the model solutions: concentrations may neither become negative nor cell populations increase to infinity, etc. Once a qualitatively robust, physiologically plausible model is obtained, it can substantially help in both the individualization of therapy in a single subject and in the introduction of new immunosuppressive (multi-pharmacological) protocol, both from safety and efficacy perspectives. Recent experiences have indeed suggested that the possibility of studying drug pharmacokinetics and pharmacodynamics through modeling techniques (*in silico*) may greatly reduce the need for animal and cellular models (Mager and Jusko, 2008), as well as the discomfort and risks associated with extensive human experimentation. In order to

work in concrete, however, the modeling approach requires a tight interconnection of mathematical constructs and physiological knowledge.

The model of the immunological response to organ transplantation described in the present work uses a system of ODE to describe the dynamics of fundamental types of antigen, together with the corresponding specific T-lymphocyte cell populations. The dynamics of the several types of antigen and lymphocytes are coupled through the effects of immunosuppressive therapy, acting equally on all types of T-cells. The continuous entry of environmental antigen (E) maintains a basal T-cell activation level in the absence of the allograft, before transplantation. Upon transplantation, two types of graft-associated antigen classes are introduced in the organism of the recipient: regenerating and non-regenerating. Regenerating antigen (L) determines the continuation over time of the immune system activation, leading to possible chronic rejection. Non-regenerating antigen has been considered under two species, given the need to consider the time delay introduced by activation: directly presented antigen (U), which stimulates T-cell clonal expansion rapidly; and non-processed, indirectly activated antigen (Y), which upon indirect presentation is converted to processed (active) antigen Z.

In this representation, some simplifications have been deliberately introduced. Among these, no discrimination has been made concerning the different T-cell types: T-lymphocytes can be either naive or activated; once activated, they differentiate into cells with specific roles (mainly helper and cytotoxic); in the present model, however, the global class of T-cells is considered, representing the response to transplantation of the immune system as a whole. The consideration of different cellular types, besides T-Lymphocytes of CD4 and CD8 class, would in fact be helpful in refining the description of the chain of events involved in the inflammatory response. Other cells of the immune system (*e.g.* antigen presenting cells, B-lymphocytes, macrophages etc.) as well as cytokines (responsible of cell proliferation, signaling and recruiting inflammation agents) are also involved in the rejection mechanisms. In the present work the need to limit model complexity has prompted the decision of representing only the cellular compartment (T-lymphocytes) most representative of solid organ rejection reaction, which directly increases in response to incoming antigen and triggers the rejection response. While the inflammatory response is not followed in detail, a measure of the inflammatory damage to the organ is however represented by the amount of circulating L antigen, assumed to be proportional to the tissue mass of living

allograft.

Another simplification consists in not representing explicitly the increased overall T-cell production occurring in the presence of inflammation. When an inflammatory process is ongoing (*e.g.* during rejection) lymphoid organs are stimulated to increase cell production non-specifically. These mechanisms are poorly understood and the actual increase in competent T-cells may not be so high as to substantially modify the response: for this reason, a constant T-cell production was assumed (Kc , equation 2).

The proposed model describes therefore the main processes taking place in the recipient immediately before and for a long time after transplantation. The pre-existing equilibrium state, characterized by constant environmental antigen and T-cells level, is dramatically perturbed by the entry of a large amount of alloantigens. If no therapy is used, T-cell expansion is determined by the presence of the antigen and the organ is rejected. Different amounts of immunosuppressive treatment cause different degrees of T-cell proliferation or clonal expansion impairment. The administration of therapy limits the immunological aggression towards the organ and the natural ability of the allograft to reproduce makes it so that an equilibrium is attained at a non-zero level of remaining allograft tissue. This is the potentially most useful area of application of future versions of the presented model, which will incorporate, besides a general biological description of the immune response, also a precise quantification of the applicable pharmacokinetics (possibly depending on the patient or on patient subgroups). Immunosuppressive therapy is very invasive and the substantial risks of potentially severe side effects have been widely discussed (Humar and Michael, 2006; Morelon and Touraine, 2007). Models for therapy improvement (*e.g.* drug dosage) have been proposed, so far only considering single aspects of the therapy (*e.g.* plasma drug concentrations) or focusing on the action of a single specific drug (Ruggeri and Martinelli, 2000; Wu et al., 2005). The model presented here has instead the aim to frame drug kinetics and effects within the immune system biology, even if the current description of it is rather elementary. The somewhat empirical therapy adjustments, which at the moment are based on organ function damage indicators and drug level monitoring, may therefore be complemented, using a model similar to the one presented here, by a quantitative systemic assessment of the likely impact of posology alterations, considering therapy effects in the context of patient individual characteristics and immune system status. Once a robust biological model is in place, it becomes relatively easy to incorporate the

effect of different drugs. It will therefore be possible to express the suppression of clonal expansion, a greater mortality of T-lymphocytes, or even a generalized action in suppressing the inflammatory response (as may happen when administering corticosteroids). The problem here will not be as much in introducing the specific actions of the array of available therapy schemes, commonly used in clinical practice, but rather in representing with some degree of accuracy those side effects, which make it undesirable to simply increase without bounds the dosage of immediately useful agents. It will become possible, in this way, to support the decision-making of the attending physician or surgeon, who has to choose a reasonable compromise between immediate therapeutic effect and long-term complications.

The model presented in this work has been developed with the aim of allowing the eventual representation of different mechanisms of action, hence of the effects, of different classes of immunosuppressive drugs. Mechanisms can differ either from the molecular or the cellular viewpoint. There are different steps, along the pathway of T-lymphocyte activation, at which drugs can act (resting state, early activation, late activation and proliferation). Polyclonal anti-lymphocyte antibodies act at the resting state. Calcineurine inhibitors (cyclosporine, tacrolimus) act in the early activation pathway, so they have the same inhibition mechanism from the cellular viewpoint. However, cyclosporine and tacrolimus have different chemical structure, and act with different mechanisms at the molecular level. It has in fact been reported that tacrolimus is more effective than cyclosporine, it is used in smaller concentrations, and there are differences in their side effects (for instance, tacrolimus is diabetogenic (Marchetti 2004), while cyclosporine induces gingival hyperplasia (Gonalves et al., 2008)). Monoclonal antibodies and rapamycin (TOR) inhibitors act in the late activation step. Antiproliferative drugs (azathioprine and mycophenolate acid) act on the last step of the activation pathway. Corticosteroids have a very different mechanism of action in that they do not inhibit T-cell production, but they act non-specifically on the inflammatory process, preventing organ failure without directly acting on T-cell dynamics (Taylor et al., 2005). Moreover, the current model can be modified by explicitly representing different steps of the activation pathway as well as focalizing on molecular aspects for a better description of different pharmaceutical action mechanisms.

The mathematical formulation of the model may appear somewhat abstract. The same model could in fact have been expressed by writing extensively all different scalar differential equations defining the behavior of

each single antigen and each single T-cell type. Apart from the fact that such a long-hand formulation would have required writing ten equations in place of Eqs. 1 and 2, the use of matrix notation allows a general expression of the interaction among the several antigenic and cellular types. While such interaction is, for the present model, limited to the production of Z antigen from Y antigen due to indirect presentation, there is reason to suppose that in the future the number of types of antigen and corresponding T-cells might be increased or that precursor cells might be introduced: these modifications will be accommodated by merely increasing the dimension of the matrices (K_{XAC} , K_{XA} , K_{CAC} , K_{XCA}), and introducing off-diagonal elements as appropriate, without any change to the overall model structure. For instance, while most antiproliferative drugs would increase (to different degrees) the value of k_{cac} , monoclonal antibodies would increase k_{xc} and k_{xa} .

The graphs, reporting the time course of the different types of antigen and corresponding T-cell populations, show that the model captures well the expected behavior of the transplanted organ mass and of the immune system reaction, under the three scenarios of no therapy, moderate therapy and aggressive therapy. From the graphs it is evident that is not easy to find the right drug dosage. It can be seen that when the drug dose is moderate T-cell levels are adequate to prevent opportunistic infections, but the L-antigen level, corresponding to the viable graft tissue, is rather low. Conversely, at a drug concentration effective in maintaining the entire transplanted tissue mass, the T-cell population is suppressed excessively and the risk of complications would appear to become substantial. The model therefore predicts that single drug therapy is inadequate to safely prevent graft rejection, in accord with the clinical experience so far accumulated. A perfect situation might not exist, but the theoretical exploration of drug combinations and of non-constant therapy schemes could be one way to obtain useful indications for the biological experimentation of novel therapeutic protocols.

The main limitations of the current model invest both the detail of therapeutic manipulations it describes and the plausibility of the represented biology. While the model, as discussed above, can be easily and naturally extended to account for more than the single pharmaceutical agent (F) incorporated so far, there are in fact important aspects of the immunological response to transplantation which have not yet been tackled. One such is the description of the Graft *versus* Host Response, which is of great importance in explaining the events following transplantation of lymphoid tissue (like bone marrow transplants), particularly after massive immunosuppres-

sion of the recipient before the operation: for this reason, the present model should be considered appropriate only for solid organ transplants (liver, kidney, pancreas, heart). Another area where greater biological detail would be useful is that of the description of the chain of events in the inflammatory process which underlie the clinical features of chronic rejection.

In conclusion, the present work proposes a first mathematical model of the cellular immune response to solid organ transplantation, addressing both acute and chronic rejection. The model's mathematical behavior is consistent with known physiology and accounts quantitatively for long-term variations in immune status and allograft survival. The model can be naturally adapted to the representation of different therapeutic regimens and may offer useful indications for the optimization of therapy protocols in the transplanted patient.

Appendix - Qualitative behavior of the solution

In order to provide a basic analysis concerning the qualitative behavior of the solutions, system (1-2) can be written according to the following mathematical formalism:

- environmental antigen subsystem:

$$\begin{aligned}\frac{dE}{dt} &= K_e - k_{xec}C_eE - k_{xe}E \\ \frac{dC_e}{dt} &= k_c + (K_{CAC}(F) - k_{xca})EC_e - k_{xc}C_e\end{aligned}\quad (23)$$

- graft antigen subsystem:

$$\begin{aligned}\frac{dL}{dt} &= L_{regmax} \frac{L}{k_{1/2} + L} + L_\tau \delta(t - t_\tau) - k_{xlc}C_lL - k_{xl}L \\ \frac{dC_l}{dt} &= k_c + (K_{CAC}(F) - k_{xca})LC_l - k_{xc}C_l\end{aligned}\quad (24)$$

- allograft antigen directly presented subsystem:

$$\begin{aligned}\frac{dU}{dt} &= U_\tau \delta(t - t_\tau) - k_{xuc}C_uU - k_{xu}U \\ \frac{dC_u}{dt} &= k_c + (K_{CAC}(F) - k_{xca})UC_u - k_{xc}C_u\end{aligned}\quad (25)$$

- allograft antigen not directly presented subsystem:

$$\begin{aligned}\frac{dY}{dt} &= Y_\tau \delta(t - t_\tau) - k_{xy}Y - k_{zy}Y \\ \frac{dZ}{dt} &= -k_{xzc}C_zZ - k_{xz}Z + k_{zy}Y \\ \frac{dC_z}{dt} &= k_c + (K_{CAC}(F) - k_{xca})ZC_z - k_{xc}C_z\end{aligned}\quad (26)$$

Note that they consist of four independent subsystems, each driven by the common input given by the drug concentration F , which evolves according to a step-wise trajectory:

$$F(t) = \begin{cases} 0, & t < t_\tau \\ \bar{F} = \frac{k_{xf}}{k_f}, & t \geq t_\tau \end{cases}\quad (27)$$

According to the step-wise feature of $F(t)$ the qualitative analysis will be done by taking into account the system before and after the drug assumption (which is assumed contemporary to the graft transplantation).

Lemma 1 Each state component of the four subsystems endowed with a physiological initial condition (all positive components), admits positive evolutions, $\forall t \geq 0$.

Proof Consider subsystem (23) and $E(0) > 0$. Due to the continuity of both $E(t)$ and dE/dt , the solution $E(t)$ would become non-positive if there existed a time instant $\bar{t} > 0$ such that $E(\bar{t}) = 0$ and $E(t) > 0$ for any $0 \leq t < \bar{t}$. Then, necessarily, $\left. \frac{dE}{dt} \right|_{t=\bar{t}} \leq 0$, which is a contradiction because:

$$\left. \frac{dE}{dt} \right|_{t=\bar{t}} = K_e - k_{xec}C_e(\bar{t})E(\bar{t}) - k_{xe}E(\bar{t}) = K_e > 0. \quad (28)$$

According to the same reasoning, it clearly appears that also $C_e(t)$ never vanishes. The same approach can be repeated for the other three subsystems.

Let us split the qualitative behavior analysis into *before* and *after* the graft transplantation. A reasonable assumption is to set $K_{CAC}(0) = k_{cacF} > k_{xca}$, as T-cell proliferation due to antigen stimulation is predominant in respect of T-cell death due to antigen contact. That means: before the simultaneous graft transplantation and drug administration, there is a positive balance between the lymphocytes proliferation/elimination induced by the corresponding antigens; on the other hand, after the graft transplantation, the drug administration makes it so that such a balance may be reduced or even become negative. In the following, in order to make more readable the equations, we will denote:

$$K_{ca}^{pre} = k_{cacF} - k_{xca} > 0 \quad \text{and} \quad K_{ca}^{after} = k_{cacF}e^{-\lambda\bar{F}} - k_{xca}. \quad (29)$$

Lemma 2 Before the graft transplantation and drug administration (i.e. $t < t_\tau$):

- i) there exists a unique positive, locally asymptotically stable equilibrium point for the environmental antigen subsystem (23);
- ii) if the following condition holds true among the coefficients of the graft antigen subsystem (24):

$$\frac{L_{regmax}}{k_{xlc}k_{1/2}} - \frac{k_{xl}}{k_{xlc}} > \frac{k_c}{k_{xc}} \quad (30)$$

then, there exists a pair of non-negative equilibrium points: one with $L = 0$ and $C_l = k_c/k_{xc} > 0$ unstable; the other, with $L > 0$ and $C_l > 0$ locally asymptotically stable. Otherwise, there exists a unique nonnegative equilibrium point with $L = 0$ and $C_l > 0$, and it is locally asymptotically stable;

- iii) there exists a unique nonnegative, locally asymptotically stable equilibrium point ($U = 0$, $C_u = k_c/k_{xc} > 0$) for the allograft antigen directly presented subsystem (25);
- iv) there exists a unique nonnegative, locally asymptotically stable equilibrium point ($Y = 0$, $Z = 0$, $C_z = k_c/k_{xc} > 0$) for the allograft antigen not directly presented subsystem (26);

Proof i) The equilibrium points of (23) satisfy the following algebraic equations:

$$\begin{aligned} K_e &= k_{xec}C_eE + k_{xe}E \\ k_c + K_{ca}^{pre}C_eE &= k_{xc}C_e \end{aligned} \quad (31)$$

from which it follows that the steady state of C_e satisfies the following second order equation:

$$k_{xec}k_{xc}C_e^2 + (k_{xe}k_{xc} - k_{xec}k_c - K_eK_{ca}^{pre})C_e - k_{xe}k_c = 0 \quad (32)$$

Since the second and zero order coefficients are positive and negative respectively, both solutions are real: one positive, the other negative, regardless to the sign of the first order coefficient (see, e.g., Franklin et al., 1994; Gantmacher 1959; Routh 1877). Thus we have a unique real positive solution for C_e . As a matter of fact, by substituting the real positive solution into the first equation of (31) we have a unique positive solution also for E :

$$E = \frac{K_e}{k_{xec}C_e + k_{xe}} > 0. \quad (33)$$

As far as the local stability analysis, let us compute the Jacobian matrix:

$$J_e = \begin{bmatrix} -k_{xec}C_e - k_{xe} & -k_{xec}E \\ K_{ca}^{pre}C_e & K_{ca}^{pre}E - k_{xc} \end{bmatrix}, \quad (34)$$

from which the characteristic polynomial is:

$$\begin{aligned} d_e(\lambda) &= \lambda^2 + (k_{xec}C_e + k_{xe} - K_{ca}^{pre}E + k_{xc})\lambda + (k_{xc} - K_{ca}^{pre}E)k_{xe} + k_{xc}k_{xec}C_e \\ &= \lambda^2 + (k_{xec}C_e + k_{xe} + k_c/C_e)\lambda + k_{xe}k_c/C_e + k_{xc}k_{xec}C_e : \end{aligned} \quad (35)$$

all positive coefficients means both the roots have positive real part, that means: local asymptotic stability of the equilibrium point.

ii) The equilibrium points of (24) satisfy the following algebraic equations:

$$\begin{aligned} L_{regmax} \frac{L}{k_{1/2} + L} &= k_{xlc} C_l L + k_{xl} L \\ k_c + K_{ca}^{pre} C_l L &= k_{xc} C_l \end{aligned} \quad (36)$$

A trivial solution is clearly given by $L = 0$ and $C_l = k_c/k_{xc} > 0$. Further real positive solutions come from the intersection of the curves in the (L, C_l) plane:

$$\begin{aligned} C_l &= \frac{L_{regmax}}{k_{xlc}(k_{1/2} + L)} - \frac{k_{xl}}{k_{xlc}} \\ C_l &= \frac{k_c}{k_{xc} - K_{ca}^{pre} L} \end{aligned} \quad (37)$$

As it appears from figure 3 a positive intersection occurs only if condition (30) is satisfied. Otherwise there will be no intersection of both positive values for L and C_l .

As far as the local stability analysis, the Jacobian matrix of subsystem (24) is:

$$J_l = \begin{bmatrix} \frac{L_{regmax} k_{1/2}}{(k_{1/2} + L)^2} - k_{xlc} C_l - k_{xl} & -k_{xlc} L \\ K_{ca}^{pre} C_l & K_{ca}^{pre} L - k_{xc} \end{bmatrix}. \quad (38)$$

In case of $L = 0$ and $C_l = k_c/k_{xc}$, the Jacobian matrix becomes triangular, with the eigenvalues:

$$\lambda_1 = \frac{L_{regmax}}{k_{1/2}} - \frac{k_{xlc} k_c}{k_{xc}} - k_{xl}, \quad \lambda_2 = -k_{xc} < 0. \quad (39)$$

Thus, if condition (30) holds true, the eigenvalue λ_1 is positive and the above mentioned equilibrium point is unstable; otherwise, the unique equilibrium point $L = 0$, $C_l = k_c/k_{xc}$ is locally asymptotically stable, being both the eigenvalues real negative. Now, let us assume (30) holds true and let us compute the characteristic polynomial for the strictly positive equilibrium point:

$$\begin{aligned} d_l(\lambda) &= \lambda^2 + \left(k_{xlc} C_l + k_{xl} - \frac{L_{regmax} k_{1/2}}{(k_{1/2} + L)^2} - K_{ca}^{pre} L + k_{xc} \right) \lambda \\ &\quad + (k_{xc} - K_{ca}^{pre} L) \left(k_{xl} + k_{xlc} C_l - \frac{L_{regmax} k_{1/2}}{(k_{1/2} + L)^2} \right) + K_{ca}^{pre} C_l k_{xlc} L \end{aligned} \quad (40)$$

According to the algebraic equations (36) the first- and zero-order coefficients of $d_l(\lambda)$ become:

$$\frac{k_c}{C_l} + \frac{L_{regmax}L}{(k_{1/2} + L)^2} > 0 \quad (41)$$

$$\frac{k_c}{C_l} \cdot \frac{L_{regmax}L}{(k_{1/2} + L)^2} + K_{ca}^{pre}C_l k_{xlc}L > 0 \quad (42)$$

respectively. Since they are both positive, both the roots of $d_l(\lambda)$ have negative real part, and ensure the local asymptotic stability of the equilibrium point.

iii) The equilibrium points of (25) satisfy the following algebraic equations:

$$\begin{aligned} k_{xuc}C_uU + k_{xu}U &= 0 \\ k_c + K_{ca}^{pre}C_uU &= k_{xc}C_u \end{aligned} \quad (43)$$

A trivial solution is clearly given by $U = 0$ and $C_u = k_c/k_{xc} > 0$. No further physically meaningful (i.e. real positive) solutions occur. The Jacobian matrix is:

$$J_u = \begin{bmatrix} -k_{xuc}C_u - k_{xu} & -k_{xuc}U \\ K_{ca}^{pre}C_u & K_{ca}^{pre}U - k_{xc} \end{bmatrix}. \quad (44)$$

If we compute J_u in $U = 0$ it readily comes that both the eigenvalues are real negative, that means the equilibrium point is locally asymptotically stable.

iv) The equilibrium points of (26) satisfy the following algebraic equations:

$$\begin{aligned} k_{xy}Y + k_{zy}Y &= 0 \\ k_{xzc}C_zZ + k_{xz}Z &= k_{zy}Y \\ k_c + K_{ca}^{pre}C_zZ &= k_{xc}C_z \end{aligned} \quad (45)$$

A trivial solution is clearly given by $Y = 0$, $Z = 0$ and $C_z = k_c/k_{xc} > 0$. No further physically meaningful (i.e. real positive) solutions occur. The Jacobian matrix is:

$$J_{yz} = \begin{bmatrix} -k_{xy} - k_{zy} & 0 & 0 \\ k_{zy} & -k_{xzc}C_z - k_{xz} & -k_{xzc}Z \\ 0 & K_{ca}^{pre}C_z & K_{ca}^{pre}Z - k_{xc} \end{bmatrix}. \quad (46)$$

If we compute J_{yz} in $Y = 0$ and $Z = 0$ it readily comes that the three eigenvalues are real negative, that means the equilibrium point is locally asymptotically stable.

Now we consider the system behavior after the simultaneous graft transplantation and drug administration. Besides the reset of three of the state variables L , U and Y whose amount is instantaneously increased of the quantities L_τ , U_τ , U_τ , respectively, we have to distinguish between the two cases of $K_{ca}^{after} > 0$ and $K_{ca}^{after} < 0$. According to the former case, the mathematical analysis does not formally change and the qualitative results of Lemma 5 are still valid. Of course, the steady state solutions after the drug administration will move in the state space, but they will not change in number nor will their stability feature change. On the other hand, if the drug administration makes it so that the net balance between proliferation and elimination changes in sign, the mathematical analysis needs to be restated.

Lemma 3 Assume that $K_{ca}^{after} > 0$ after the graft transplantation and drug administration (i.e. $t \geq t_\tau$). Then, the qualitative behavior of the subsystems (23), (25) and (26) as stated in Lemma 5, items i), iii), iv) are valid the same.

Proof The three items of Lemma 5 will be proved.

i) As far as the equilibrium points of (23), consider the algebraic system (31) and substitute $K_{ca}^{pre} > 0$ with $K_{ca}^{after} < 0$, from which it follows that the steady state of C_e satisfies a second order equation with the second and zero order coefficients positive and negative respectively: both solutions are real, one positive, the other negative, regardless to the sign of the first order coefficient. Thus we have a unique real positive solution for C_e to which, by substitution, a unique positive solution occurs also for E . As far as the local stability analysis, the characteristic polynomial does not explicitly depend on K_{ca}^{after} (as computed in (35)), thus the same results before the drug administration can be written: local asymptotic stability for the equilibrium point.

iii-iv) The qualitative behavior is not seem affected by the change of sign of $K_{ca}^{after} < 0$.

Lemma 4 Assume that $K_{ca}^{after} > 0$ after the graft transplantation and drug administration (i.e. $t \geq t_\tau$). Then, if condition (30) holds true among the coefficients of the graft antigen subsystem (24), there exists a pair of non-negative equilibrium points: one with $L = 0$ and $C_l = k_c/k_{xc} > 0$ unstable; the other, with $L > 0$ and $C_l > 0$ locally asymptotically stable, provided that the following further condition holds true:

$$\frac{k_c}{C_l} \cdot \frac{L_{regmax}}{(k_{1/2} + L)^2} + K_{ca}^{after} C_l k_{xlc} > 0 \quad (47)$$

where C_l and L are the strictly positive steady state solutions. If condition

(30) does not hold true, there exists a unique nonnegative equilibrium point with $L = 0$ and $C_l > 0$, and it is locally asymptotically stable;

Proof Consider the algebraic system (36) and substitute $K_{ca}^{pre} > 0$ with $K_{ca}^{after} < 0$. A trivial solution is clearly given by $L = 0$ and $C_l = k_c/k_{xc} > 0$. Further real positive solutions come from the intersection of the curves (37) in the (L, C_l) plane. As it appears from figure 4 a positive intersection occurs only if condition (30) is satisfied. Otherwise there will be no intersection of both positive values for L and C_l .

As far as the local stability analysis, the Jacobian matrix of subsystem (24) dose not change (w.r.t. the case before the drug assumption) if computed for the equilibrium with $L = 0$: thus, if condition (30) holds true, one of the two eigenvalues is positive and the equilibrium point is unstable; otherwise, the unique equilibrium point $L = 0$, $C_l = k_c/k_{xc}$ is locally asymptotically stable. Now, let us assume (30) holds true. The characteristic polynomial for the second positive equilibrium point becomes:

$$d_l(\lambda) = \lambda^2 + \left(k_{xlc}C_l + k_{xl} - \frac{L_{regmax}k_{1/2}}{(k_{1/2}+L)^2} + K_{ca}^{after}L + k_{xc} \right) \lambda + (k_{xc} - K_{ca}^{after}L) \left(k_{xl} + k_{xlc}C_l - \frac{L_{regmax}k_{1/2}}{(k_{1/2}+L)^2} \right) + K_{ca}^{after}C_l k_{xlc}L \quad (48)$$

According to the algebraic equations (36) the first- and zero-order coefficients of $d_l(\lambda)$ become:

$$\frac{k_c}{C_l} + \frac{L_{regmax}L}{(k_{1/2} + L)^2} > 0 \quad (49)$$

$$\frac{k_c}{C_l} \cdot \frac{L_{regmax}L}{(k_{1/2} + L)^2} + K_{ca}^{after}C_l k_{xlc}L \quad (50)$$

respectively. If condition (47) holds true, then the characteristic polynomial has all positive coefficients, that means its roots have negative real part and the equilibrium point is locally asymptotically stable.

Remark 1 It should be interesting to discuss whether the administration of sufficiently large amounts of immunosuppressive drug has the ability to essentially annihilate the proliferation of competent T-lymphocytes. If so, then k_c (reflecting drug-independent lymphocyte production) should be set to zero, eliminating the possibility of attaining a positive equilibrium, i.e. the eventual preservation of a non-negligible allograft tissue amount in the presence of massive immunosuppression. Viceversa, if k_c were set to a positive quantity, even the most intense immunosuppression would not be able to completely eliminate T-lymphocyte production, and, in the case of

a substantially small allograft regenerative ability L_{regmax} , eventual disappearance of the allograft would occur.

Remark 2 Assume the case of $K_{ca}^{after} < 0$ and both conditions (30) and (47) hold true: we still have in any case bounded solutions. Indeed, assume $\limsup_{t \rightarrow +\infty} L(t) = +\infty$. Then, due to continuity, $\exists \{t_n\} \subset [t_\tau, +\infty)$:

$$\lim_{n \rightarrow +\infty} t_n = +\infty, \quad \lim_{n \rightarrow +\infty} L(t_n) = +\infty, \quad \text{with} \quad \left. \frac{dL}{dt} \right|_{t=t_n} \geq 0. \quad (51)$$

But:

$$\left. \frac{dL}{dt} \right|_{t=t_n} = L_{regmax} \frac{L(t_n)}{k_{1/2} + L(t_n)} - k_{xlc} C_l(t_n) L(t_n) - k_{xl} L(t_n) \mapsto -\infty, \quad (52)$$

which is a contradiction, so $\limsup_{t \rightarrow +\infty} L(t) < +\infty$. On the other hand, assume $\limsup_{t \rightarrow +\infty} C_l(t) = +\infty$. Then, due to continuity, $\exists \{t_n\} \subset [t_\tau, +\infty)$:

$$\lim_{n \rightarrow +\infty} t_n = +\infty, \quad \lim_{n \rightarrow +\infty} C_l(t_n) = +\infty, \quad \text{with} \quad \left. \frac{dC_l}{dt} \right|_{t=t_n} \geq 0. \quad (53)$$

But:

$$\left. \frac{dC_l}{dt} \right|_{t=t_n} = k_c + K_{ca}^{after} L(t_n) C_l(t_n) - k_{xc} C_l(t_n) \mapsto -\infty, \quad (54)$$

which is a contradiction, so $\limsup_{t \rightarrow +\infty} C_l(t) < +\infty$.

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