

Complex Multicellular Systems and Immune Competition: New Paradigms Looking for a Mathematical Theory *

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This chapter deals with the modelling and simulation of large systems of interacting entities whose microscopic state includes not only geometrical and mechanical variables (typically position and velocity), but also biological functions or specific activities. The main issue looks at the development of a biological mathematical theory for multicellular systems. The first part is devoted to the derivation of mathematical structures to be properly used to model a variety of

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biological phenomena with special focus on the immune competition. Then, some specific applications are proposed referring to the competition between neoplastic and immune cells. Finally, the last part is devoted to research perspectives towards the objective of developing a mathematical-biological theory. A critique is presented of what has already been achieved towards the above target and what is still missing with special focus on multiscale systems.

1 Introduction

This chapter deals with the challenging objective of developing a mathematical theory of biological multicellular systems, with special focus on the competition between the immune system and cancer cells. The overall project does not simply deal with the design of a mathematical model, but it will lead towards a robust mathematical description of biological activities. This is a fascinating perspective that is worth pursuing despite its enormous conceptual difficulties. Towards this goal, the heuristic experimental approach, which is the traditional method of investigation in biological sciences, should be gradually integrated with new methods and paradigms generated by progressive interaction with mathematical sciences.

A mathematical description of living matter is conceptually different from the description of inert matter. As observed by May (2004), mathematical theory and experimental investigation have always marched together in the physical sciences. Mathematics has been less intrusive in the life sciences, possibly because they have been until recently descriptive, lacking the invariance principles and fundamental natural constants of physics.

Nevertheless, several hints to interdisciplinary approaches have been offered. Hartwell et al. (1999) point to the fact that although living systems obey the laws of physics and chemistry, the notion of function and purpose differentiates biology from other natural sciences. Reproduction, competition, cell cycle, ability to communicate with other entities are features absent in classic Newtonian mechanics. Furthermore, the macroscopic features of a system constituted by million of cells shows only the output of cooperative behaviour and not the activities of the various cells. Remarkably similar concepts are proposed by Reed (2004) that consider the issue from the view point of applied mathematics.

Hartwell's suggestions are not limited to general speculations, but provide a theory of functional modules that actually contributes to the development of a mathematical theory for biological systems and that will be used in the present study. A functional module is defined as a discrete entity whose function is separable from those of other modules.

The present approach will use the mathematical kinetic theory for living particles in order to describe complex multicellular systems dealing with cell expansions, cell death and immune supervision. The analysis will be developed at the

cellular scale, as an intermediate between the subcellular and macroscopic scales. Our kinetic theory for living systems is based on functional modules and will be implemented using game theory to describe cell interactions leading to selective expansions and death.

2 Conceptual Lines Towards a Mathematical Biological Theory

In the present chapter we use the mathematical kinetic theory for living particles in order to describe complex multicellular systems dealing with cell expansions, cell death and immune supervision. The modelling is developed at the cellular scale, as an intermediate between the subcellular and macroscopic scales.

The traditional approach of the mathematical sciences to biology generates mathematical models which we separate from a mathematical theory. Models may even occasionally reproduce specific aspects of biological phenomena, while they are rarely able to capture their essential features. On the other hand, a mathematical theory provides structures rigorously deduced on the basis of mathematical assumptions whose validity is consistent with a large variety of physical systems.

Our kinetic theory attempts to describe the statistical evolution of large systems of interacting particles whose microscopic state includes activity, a variable related to the expression of biological function. In addition to geometrical and mechanical variables, it is the kinetic theory for active particles, while admittedly, additional work is needed to develop a comprehensive mathematical-biological theory.

In the framework of the mathematical kinetic theory we will evaluate the statistical distribution of the activity (defined as state) of the cells forming the different populations considered in the model. The third step involves in the derivation of mathematical equations describing the evolution of the cell state. This will be pursued through conservation equations describing the numerical modulation of cells denoted by a defined state.

In the physical sciences the analog of a multi-cellular system is a gas mixture of particles. Usually this involves a small number of components, whereas a biological system is commonly denoted by a *game* with a large number of cell populations. Interactions among particles obey the laws of classical Newtonian mechanics without amplification and death events. By contrast, the latter are the hallmarks of cellular systems. Detailed information on how cell interactions are regulated by signals emitted or perceived by the cells and transduced to the nucleus, where they participate in the modulation of cell activities, is provided by biological studies. As a consequence, these interactions the cell acquires a new state.

Mathematical models describe these cell activities with ordinary differential

equations or Boolean networks, while multicellular systems are modelled by non-linear integro-differential equations similar to those of nonlinear kinetic theory (the Boltzmann equation), or by individual-based models which give rise to a large set of discrete reactions. On the other hand, applied mathematics attempts to deal with the biological complexity of cell behaviour by isolating a few activities and studying them through differential equations. However, it is not generally possible to isolate specific biological activities from the whole system under consideration. Interactions among cell subpopulations may dramatically modulate the resulting cell activity, and thus the model cannot be limited to one specific activity isolated from its cellular microenvironment.

Moreover, a mathematical theory developed at the cellular scale should retain suitable information from the lower molecular scale, while it should allow the derivation of macroscopic equations by suitable asymptotic limits related to condensation and fragmentation events. This matter is even more relevant (Belomo and Maini (2006)) considering that one of the major problems in modelling biological systems is the multiscale nature of most biological systems.

3 From Hartwell's Theory of Modules to Mathematical Structures

Our mathematical kinetic theory deals with the analysis of the evolution of multicellular systems, far from equilibrium. In dealing with the enormous difficulty in deriving a mathematical framework, reference to physical sciences provides a useful background: the state of each microscopic entity, the particle, is identified by geometrical and mechanical variables: position and orientation, as well as velocity and rotation. In our theory, the state of a cell includes specific activities which may differ among cell populations as well as common activities such as cell expansion and death.

To deal with the biological complexity, the Hartwell's theory of functional modules (1999) can be exploited. The behaviour of cell populations engaged in a defined activity should be considered as a collective whole.

Consider a large system of n interacting cell populations homogeneously distributed in space. The microscopic state of cells is identified by a scalar variable $u \in D_u$ which represents the relevant biological function expressed by cells of each population.

The biological function differs from population to population, while the overall statistical representation of the system is described by the distribution functions:

$$f_i = f_i(t, u) : [0, T] \times D_u \rightarrow \mathbb{R}_+, \quad i = 1, \dots, n, \quad (1)$$

where the subscript i labels the i^{th} population, and, by definition, $dN_i = f_i(t, u) du$ denotes the number of cells, regarded as active particles, which, at time t , are in the element $[u, u + du]$ of the space of the microscopic states.

A mathematical model should describe the evolution in time of the distribution functions f_i . When these functions are obtained by solution of suitable mathematical problems, then gross averaged quantities can be computed. For instance, the local **number density** of cells is computed, under suitable integrability assumptions on f_i , as follows:

$$n_i(t) = \int_{D_u} f_i(t, u) du, \quad (2)$$

while the **activation** and the **activation densities** are, respectively, given by

$$a_i = a[f_i](t) = \int_{D_u} u f_i(t, u) du, \quad (3)$$

and

$$A_i = A[f_i](t) = \frac{a[f_i](t)}{n_i(t)}. \quad (4)$$

Analogous calculations can be developed for higher order moments.

Considering the kinetic theory for active particles Bellouquid and Delitala (2006), applied to modelling multicellular systems, the derivation of a mathematical framework suitable to describe the evolution of the distribution functions $f_i = f_i(t, u)$ appears appropriate to describe the evolution of the system under consideration. Such a framework acts as a general paradigm for the derivation of specific models generated by a detailed modelling of cellular interactions. It needs to be stressed that only a few activities that change the state of the cells are taken into account, such as:

- Stochastic modification of the microscopic state of cells due to binary interactions with other cells of the same or of different populations. These interactions are called **conservative** as they do not modify the number density of the various populations
- Genetic alteration of cells which may either increase the progression of tumor cells or even generate, by clonal selection, new cells in a new population of cancer cells with higher level of malignancy
- Proliferation or destruction of cells due to interaction with other cells of the same or of different populations. Proliferation refers to both tumor and immune cells.

The formal equation, which describes the evolution of f_i , is obtained by the balance of particles in the elementary volume of the state of microscopic state. A technical derivation of that proposed in Bellouquid and Delitala (2005) and (2006) is as follows:

$$\frac{\partial}{\partial t} f_i(t, u) = J_i[f](t, u) = C_i[f](t, u) + P_i[f](t, u) + D_i[f](t, u), \quad (5)$$

where the right side term models the flow, at the time t , into the elementary volume $[u, u + du]$ of the state space of the i^{th} population due to transport and interactions. In details:

- $C_i[f](t, u)$ models the flow, at time t , into the elementary volume of the state space of the i^{th} population due to conservative interactions:

$$C_i[f](t, u) = \sum_{j=1}^n \eta_{ij} \int_{D_u} \int_{D_u} \mathcal{B}_{ij}(u_*, u^*; u) f_i(t, u_*) f_j(t, u^*) du_* du^* - f_i(t, u) \sum_{j=1}^n \eta_{ij} \int_{D_u} f_j(t, u^*) du^*, \quad (6)$$

where η_{ij} is the encounter rate, referred to encounters of a **candidate particle**, with state u_* in the i^{th} population, and a **field particle**, with state u^* in the j^{th} population. $\mathcal{B}_{ij}(u_*, u^*; u)$ denotes the probability density that the candidate particles fall into the state u remaining in the same populations. Conservative equations modify the microscopic state, but not capacity to produce a clonal expansion.

- $P_i[f](t, u)$ models the flow, at time t , into the elementary volume of the state space of the i^{th} population due to proliferating interactions with transition of population:

$$P_i[f](t, u) = \sum_{h=1}^n \sum_{k=1}^n \eta_{hk} \int_{D_u} \int_{D_u} \mu_{hk}^i(u_*, u^*; u) f_h(t, u_*) f_k(t, u^*) du_* du^*, \quad (7)$$

where $\mu_{hk}^i(u_*, u^*; u)$ models the net proliferation into the i^{th} population, due interactions, which occur with rate η_{hk} , of the **candidate particle**, with state u_* , of the h^{th} population and the **field particle**, with state u^* , of the k^{th} population.

- $D_i[f](t, u)$ models the net flow, at time t , into the elementary volume of the state space of the i^{th} population due to proliferating and destructive interactions without transition of population:

$$D_i[f](t, u) = \sum_{j=1}^n \eta_{ij} \int_{D_u} \mu_{ij}(u_*, u^*, u) f_i(t, u_*) f_j(t, u^*) du_* du^*, \quad (8)$$

where $\mu_{ij}(u, u^*)$ models net flux within the same population due to interactions, which occur with rate η_{ij} , of the **test particle**, with state u , of the i^{th} population and the **field particle**, with state u^* , of the j^{th} population.

Substituting the above expression into (5), yields:

$$\begin{aligned}
\frac{\partial}{\partial t} f_i(t, u) &= \sum_{j=1}^n \eta_{ij} \int_{D_u} \int_{D_u} \mathcal{B}_{ij}(u_*, u^*; u) f_i(t, u_*) f_j(t, u^*) du_* du^* \\
&- f_i(t, u) \sum_{j=1}^n \eta_{ij} \int_{D_u} f_j(t, u^*) du^* \\
&+ \sum_{h=1}^n \sum_{k=1}^n \eta_{hk} \int_{D_u} \int_{D_u} \mu_{hk}^i(u_*, u^*; u) f_h(t, u_*) f_k(t, u^*) du_* du^* \\
&+ \sum_{j=1}^n \eta_{ij} \int_{D_u} \mu_{ij}(u_*, u^*, u) f_i(t, u_*) f_j(t, u^*) du_* du^*. \tag{9}
\end{aligned}$$

The above structure acts as a paradigm for the derivation of specific models, to be obtained by a detailed modelling of microscopic interactions. The modelling needs to be finalized to generate well defined expressions of the terms η , \mathcal{B} , and μ . However, following the idea of reducing complexity, each population is identified only by the ability of performing one activity only. This reduction of complexity fits well with the suggestion, due to Hartwell, to regard a population as a modulus with the ability of expressing a well defined activity. A simpler structure is obtained in absence of generation of particles in a population different from that of the interacting pairs with proliferation and destruction in the state of the test particle. In this case, the mathematical structure is as follows:

$$\begin{aligned}
\frac{\partial}{\partial t} f_i(t, u) &= \sum_{j=1}^n \eta_{ij} \int_{D_u} \int_{D_u} \mathcal{B}_{ij}(u_*, u^*; u) f_i(t, u_*) f_j(t, u^*) du_* du^* \\
&- f_i(t, u) \sum_{j=1}^n \eta_{ij} \int_{D_u} f_j(t, u^*) du^* \\
&+ f_i(t, u_*) \sum_{j=1}^n \eta_{ij} \int_{D_u} \mu_{ij}(u, u^*) f_j(t, u^*) du_* du^*. \tag{10}
\end{aligned}$$

As already mentioned, the above reduction of complexity corresponds to an interpretation of the theory of functional modules. Hence, each population is regarded as a modulus with the ability of expressing a well defined biological function.

4 A Simple Application and Perspectives

The literature on the modelling of cellular systems by the methods of mathematical kinetic theory for active particles was first proposed by us, Bellomo and Forni

(1994), and then developed by various authors as documented by, for example, Kolev (2003), (2005), Bellouquid and Delitala (2004), (2005), (2006), De Angelis and Jabin (2003), Derbel (2004). Models are related to biological theories of cell competition, see Dunn et al. (2002), Blankenstein (2005), Friedl et al. (2005).

The above literature shows how models describe the output of the competition according to an appropriate choice their parameters, of the model, which can be possibly modified by suitable therapeutical actions. The afore mentioned parameters have a well defined biological meaning and can be identified by appropriate experiments.

Specifically, the above models describe, depending on the above mentioned parameters, progression and heterogeneity phenomena (Greller et al. (1996), Nowell (2002)), as well as various aspects of immune competition. They show either the growth and blow-up of tumor cells after progressive inhibition of the immune cells, or the destruction of tumor cell density by the immune system which remains active. The output depends, not only on the proliferation ability of progressing cells, but also on the ability of immune cells to target antigens and then destroy tumor cells.

Some models include the description of therapeutical actions by adding new populations which have the ability to modify the output the competition, e.g. Kolev et al. (2005), Brazzoli and Chauviere (2006), De Angelis and Jabin (2005). In some cases immune activation, e.g. d'Onofrio (2006), and multiple therapies have to be taken into account, e.g. De Pillis et al. (2006). These papers show the flexibility of this theory as it can be applied to different models of cell expansion, differentiation and surveillance. It also permits us to assess the consequences of therapeutical interventions. In a few models, the inclusion of additional cell populations changes the final result, while in other the effects of particular immune stimulations or multiple therapies have been taken into account.

Before dealing with an illustrative application of various aspects of the above mentioned competition, it is worth developing a critical analysis of the existing literature in the field in view of further research perspectives. Specifically, let us stress that all models cited above are characterized by the following:

- i) The number of interacting cell populations is small compared with the number of cell populations and sub-populations that probably play a role *in vivo*. For instance, in the modelling of tumor-host interactions only normal, neoplastic, and immune cell populations are considered.
- ii) The derivation of all models needs a detailed description of interactions at the microscopic level. These specific models are obtained by a phenomenological interpretation, based on causality effects, of biological reality.
- iii) Therapeutical actions are modelled by addition of new populations which have the ability to activate the immune response of weakening the progression of tumor cells.

A more accurate modelling of the onset of cancer, the inclusion into the model

of additional cell populations denoted by specialized cell functions, will improve the predictive ability of the model as shown by Pappalardo et al. (2005) and Lollini et al. (2006). The significance of a model is markedly increased by its ability not only to provide a quantitative simulation of what can be experimentally observed, but also to focus on events which are not experimentally evident. Subsequent experiments could be planned to specifically assess and validate model predictions.

This section analyzes how various phenomena of interest in biology can be described by models related to the mathematical structures (9) and (10), where the various terms characterizing cell interactions are obtained by a phenomenological interpretation of physical reality. Simple models can be improved to include the description of additional events. Specifically, a relevant biological characteristic, remarked in the paper by Greller et al. (1996), is the *heterogeneity* over the activity variable, which means that biological functions are not the same for all cells, but are statistically distributed (for progression) as already discussed in the previous section.

A simple model described in what follows, referring to the mathematical structure (9), is described as the technical interpretation of the contents of the above cited book by Bellouquid and Delitala (2006). Then some developments are analyzed taking advantage of several interesting hints recently proposed by Delitala and Forni (2007) who propose a model which describes sequential genetic mutations of a neoplastic cell with increasing level of malignancy.

Consider then a system constituted by two populations whose microscopic state $u \in (-\infty, \infty)$ has a different meaning for each population:

$i = 1$: *Environmental cells* - The state u refers to *natural* state (normal stromal cells) for negative values of u ; to abnormal state, i.e. cells which have lost their differentiated state and become progressing cells, for positive values of u , with the additional ability to inhibit immune cells.

$i = 2$: *Immune cells* - Negative values of u correspond to non-activity or *inhibition*; positive values of u to *activation* and hence their ability to *contrast* the growth of tumor cells.

Before providing a detailed description of microscopic interactions, some preliminary assumptions, which reduce the complexity of the system, are stated:

H.1. The number and distribution of cells of the first population is denoted by $f_1(0, u) = f_{10}(u)$ and is supposed to be constant in time for $u \in (-\infty, 0]$, while the distribution functions of all cell populations are normalized with respect to the initial number density of that population:

$$n_{10} = \int_{-\infty}^0 f_{10}(u) du. \quad (11)$$

H.2. The distribution over the velocity variable is constant in time, therefore, the encounter rate is constant for all interacting pairs. For simplicity it will be

assumed: $\eta_{ij} = 1$, for all i, j .

H.3. The term \mathcal{B}_{ij} , related to the transition probability density, is assumed to be defined by a given delta distribution identified by the most probable output $m_{ij}(u_*, u^*)$, which depends on the microscopic state of the interacting pairs:

$$\mathcal{B}_{ij}(u_*, u^*; u) = \delta(u - m_{ij}(u_*, u^*)). \quad (12)$$

Then, after the above preliminary assumptions, a detailed description of the nontrivial interactions, assumed to play a role in the evolution of the system, is given according to the phenomenological models described in what follows, while interactions which do not affect the evolution of the system are not reported.

• **Conservative interactions**

C.1. Interactions between cells of the first population generate a continuous trend in this population towards progressing states identified by the most probable output:

$$m_{11} = u_* + \alpha_{11},$$

where α_{11} is a parameter related to the inner tendency of both a normal and mutated cell to degenerate.

C.2. The most probable output of the interaction between an active immune cell with a progressing cell is given as follows:

$$u_* \geq 0, u^* \geq 0: \quad m_{21} = u_* - \alpha_{21},$$

where α_{21} is a parameter which indicates the ability of mutated cells to inhibit immune cells.

• **Proliferating-destructive interactions**

P-D.1. Progressing cells undergo uncontrolled mitosis stimulated by encounters with non-progressing cells due to their angiogenic ability:

$$\mu_{11}(u, u^*) = \beta_{11} U_{[0, \infty)}(u) U_{(-\infty, 0]}(u^*),$$

where β_{11} is a parameter which characterizes the proliferating ability of mutated cells.

P-D.2. Active immune cells proliferate due to encounters with progressing cells, although some of them may be inhibited:

$$\mu_{21}(v, w) = \beta_{21} U_{[0, \infty)}(v) U_{[0, \infty)}(w),$$

where β_{21} is a parameter which characterizes the proliferating ability of immune cells.

P-D.3. Progressing cells are partially destroyed due to encounters with active immune cells.

$$\mu_{12}(u, u^*) = -\beta_{12}U_{[0,\infty)}(u)U_{[0,\infty)}(u^*),$$

where β_{12} is a parameter which characterizes the destructive ability of active immune cells.

Based on the above modelling of cell interactions, the evolution equation, Eq. (10) generates the following model:

$$\begin{aligned} \frac{\partial f_1}{\partial t}(t, u) &= [f_1(t, u - \alpha_{11}) - f_1(t, u)] \int_{-\infty}^0 f_1(t, u) du \\ &+ f_1(t, u) \left[\beta_{11} \int_{-\infty}^0 -\beta_{12} \int_0^{\infty} f_2(t, u) du \right] U_{[0,\infty)}(u), \quad (13) \\ \frac{\partial f_2}{\partial t}(t, u) &= \int_0^{\infty} f_1(t, u) du f_2(t, u + \alpha_{21}) U_{[0,\infty)}(u + \alpha_{21}) \\ &+ (\beta_{21} - 1) \int_0^{\infty} f_1(t, u) du f_2(t, u) U_{[0,\infty)}(u), \end{aligned} \quad (14)$$

where the stepwise function, $U_{[a,b]}(z)$ is such that: $U_{[a,b]}(z) = 1$, if $z \in [a, b]$ and $U_{[a,b]}(z) = 0$, if $z \notin [a, b]$.

The above model is characterized by five positive phenomenological parameters, which are small with respect to one:

α_{11} corresponds to the tendency of cells to acquire progression through mutations,

α_{21} corresponds to the ability of mutated cells to inhibit the active immune cells,

β_{11} corresponds to the proliferation rate of mutated cells,

β_{12} corresponds to the ability of immune cells to destroy mutated cells,

β_{21} corresponds to the proliferation rate of immune cells,

The α -parameters are related to conservative encounters, while the β -parameters are related to proliferation and destruction.

In general, it is interesting analyzing the influence of the parameters of the model and of the mathematical problem over the following two different behaviors:

i) Blow-up of progressing cells which are not sufficiently controlled by immune cells due both to the fast progression of tumor cells and to the weak proliferation of immune cells.

ii) Destruction of progressing cells due to the action of the immune system which has a sufficient proliferation rate.

Simulations may be used to analyze the role of the parameters in determining the outcome of the competition. For instance to show how different progression rates may lead to different outputs of the competition.

The above two different behaviors are visualized in Figures 1, 2, 3, and 4, according to the solution of the initial value problem for Eqs. (13)-(14) corresponding to a different selection of the parameters. In particular, Figure 1 shows how the distribution function of tumor cells moves towards greater values of progression. Correspondingly immune cells are progressively inhibited as shown in Figure 2. Simulations are obtained for the following values of the parameters: $\alpha_{11} = \alpha_{21} = 0.1$, $\beta_{11} = 0.5$, and $\beta_{12} = \beta_{21} = 0.05$.

The opposite behavior is shown in Figures 3 and 4, where the parameters related to the activities of immune cells have been selected corresponding to a higher defence ability: $\beta_{12} = 0.5$ and $\beta_{21} = 0.9$. In this case the immune activation shows ability to reduce progression of tumor cells and finally suppress them.

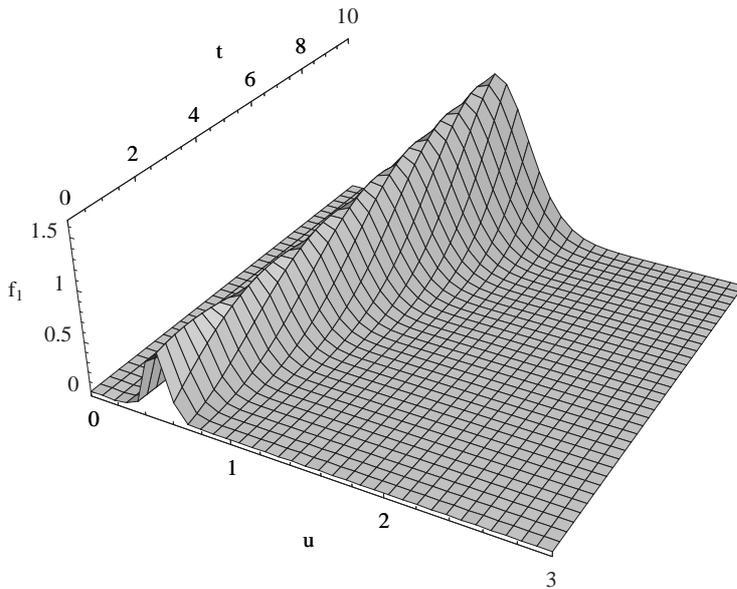


Figure 1: Heterogeneity and progression of tumor cells which increase their progression and proliferate due to their ability to inhibit immune cells. Simulations are obtained by solution of Eqs. (13)-(14) in the case of large values of α_{21} .

The mathematical model described above has been derived within the framework defined by Eq. (10) which does not include the modelling of progressive genetic instability of the tumor. It follows that the model can describe biological phenomena on a short time interval, while for longer time genetic mutations play an important role in the evolution of the system. Moreover, the model has been derived for a small number of populations (regarded as modules expressing

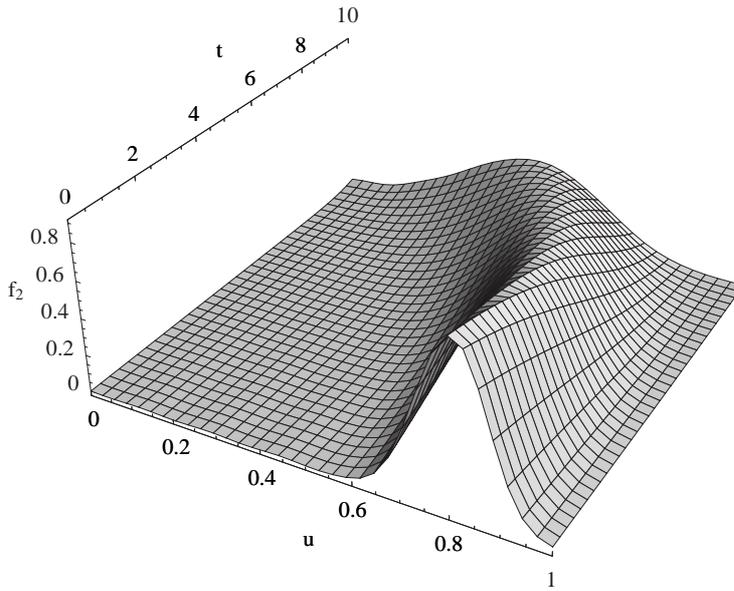


Figure 2: Progressive inhibition of immune cells. Simulations are obtained by solution of Eqs. (13)-(14) with the same parameters used for Figure 1.

a well defined biological function), so that their mathematical structure is not too complex for a computational analysis.

Definitely, the above modelling approach has to be regarded as an approximation of physical reality considering that biological functions are the output of the collective behavior of several cell populations. A relatively more refined interpretation of the theory may identify the function expressed by each population, so that their number is consistently increased. For instance, the modelling of immune cells can be developed looking at the role of each specific population rather than looking at the whole as one module only. Moreover, genetic instability in tumors favors the onset of a finite number of new populations with increasing degrees of malignancy related to subsequent genetic transitions. The downside of the above approach is that the number of parameters also increases and their identification may become very difficult or even impossible.

The above developments can be referred to Hartwell's theory of modules, as they correspond to assuming that genetic modifications are identified at different stages by discrete variables and that each population is regarded as a module which expresses the same type of biological function.

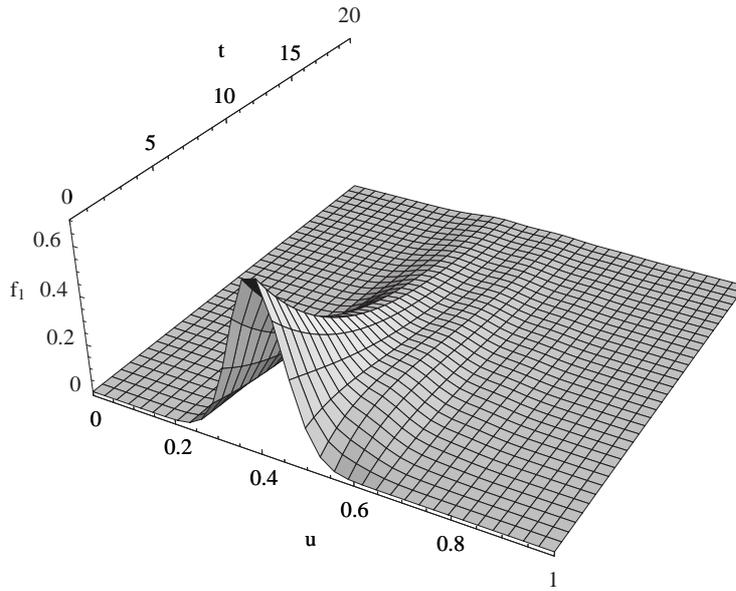


Figure 3: Heterogeneity and progressive destruction of tumor cells Eqs. (13)-(14) which decrease their progression and are progressively suppressed by immune cells. Simulations are obtained by solution of Eqs. (13)-(14) in the case of small values of α_{21} .

5 What is Still Missing for a Biological Mathematical Theory

This final section goes back to the main issue of this chapter, the assessment of additional work finalized to develop a proper biological-mathematical theory along the conceptual lines already defined. Some useful guidelines can be extracted again from Hartwell et al. (1999). Referring to the contents of the preceding sections, three items acquire a critical importance:

I The notion of function or purpose differentiate living systems in biology from those of inert matter. Biological functions have the ability to modify the conservation laws of classical mechanics and, in addition, can generate destructive and/or proliferating events.

II Biological cells contain a large number of copies, each characterized by specific functions.

III Systems in biology cannot be simply observed and interpreted at a macroscopic level. A system constituted by millions of cells shows at the macroscopic level only the output of cooperative and organized behaviors which may not, or are not,

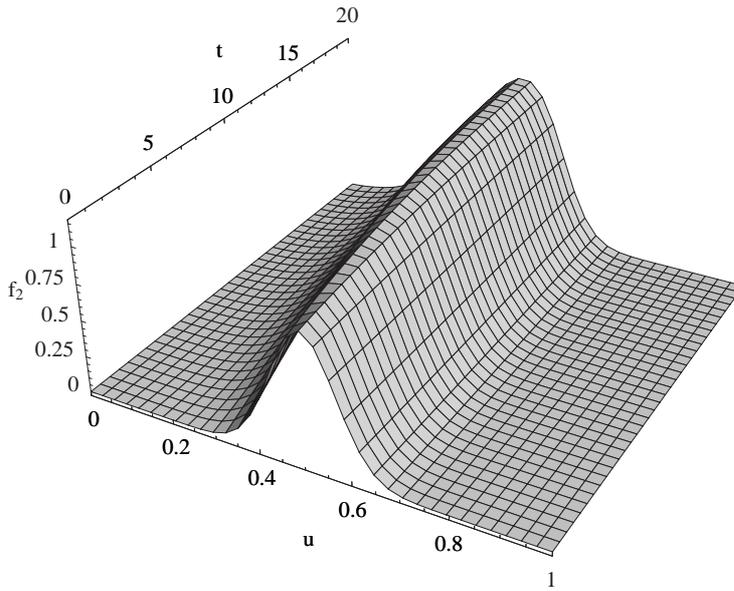


Figure 4: Immune cells remain sufficiently active. Simulations are obtained by solution of Eqs. (13)-(14) with the same parameters used for Figure 3.

singularly observed.

Developing a research activity towards the perspective we have defined means that the paradigms of the traditional approach should be replaced by new ones, while applied mathematics should not attempt to describe complex biological systems by simple paradigms and equations. This approach has often generated various unsuccessful attempts and ultimately constitutes even an effective obstacle.

Therefore, we need to verify that the mathematical structures we propose are effectively consistent, according to the above three critical items. The answer is definitely positive for the first two items, considering that biological functions are represented by the activity variables and that the application of the theory of modules can reduce the number of cell populations which effectively *play the game*. A greater (or smaller) number of populations can possibly provide a more (or less) accurate description of the system under consideration. The price to pay, when the number of populations is increased, is that additional work has to be done to assess all parameters of the model.

Moreover, both the mathematical structure and the model we propose have shown that biological events, such as proliferating and destructive interactions or

generation of new populations related to progressive genetic mutations, can be described by mathematical equations.

On the other hand, while our mathematical structure is a candidate to derive specific models, still one has to stated that a biological-mathematical theory has been created. Possibly, the afore mentioned structure refers to a mathematical theory, Bellomo and Forni (2006), considering that a new class of equations has been generated. Indeed, a rigorous framework is given for the derivation of models, when a mathematical description of cell interactions can be derived, by phenomenological interpretation, from empirical data. On the other hand, only when the above interactions are determined by a theoretical interpretation given within the framework of biological sciences, may we talk about a **biological-mathematical theory**.

In more detail, biological sciences should provide, by a robust theory, the various terms which characterize the class of equations (9):

the encounter rate η_{hk} ;

the transition probability density \mathcal{B}_{hk} ;

the population transition terms μ_{hk}^i ;

the proliferating/destructive terms μ_{hk} ;

where, in general, the above quantities may depend on the microscopic states.

An analogy can be given with the physics of classical particles whose dynamics is ruled by particle interaction models described by attractive-repulsive potentials. Newtonian mechanics provides the necessary mathematical background to describe particle interactions by attraction-repulsion potentials of the interacting particles, or by mechanical collisions which preserve mass, momentum and energy. It is worth stressing that a deep analysis of the inner structure of atoms or molecules is not necessary, but simply a theoretical description of the interaction potentials which govern pair interactions between particles. In the case of multicellular systems, the cell state includes, in addition to the mechanical state, also biological functions which have the ability of modifying their mechanical behavior. In our case, biology should contribute, by experiments and theoretical interpretations, to describe the outcome of cellular interactions. Specifically, the above four terms should be elucidated at the molecular level, i.e. at the lower scale.

Although at present such a theory is not yet available, it is well understood that the objective can be achieved only through a detailed analysis of gene expression related to biological functions at the molecular scale. Various theoretical approach have been proposed, among others Novak and Sigmund (2004), Komarova (2004), Gatenby et al. (2005), related to specific theories in the field of biological sciences, e.g. Hanahan and Weinberg (2000), Baylin and Ohm (2006), Merlo et al. (2006), Anderson et al. (2005), (2006), where biology put clearly in evidence the evolutionary and ecological aspect of cancer onset and evolution.

The various theoretical approaches known in the literature postulate probabilistic models of gene expression, while gene interactions among themselves and with the external environment should be taken into account. Considering that a robust theory is not yet available, a conjecture is proposed here to develop at the molecular scale some ideas already exploited at the cellular scale. In other words, we conjecture that the structures (9) or (10) can be used to describe the dynamics at the molecular scale to derive, out of this dynamics, the above main cellular interaction terms.

Finally, let us deal with the third critical issue. As we have seen, although the modelling and analysis has been developed at the cellular scale, looking at the lower scale appears to be necessary to recover the above interaction terms. The underlying microscopic description should provide the macroscopic description, which can be possibly observed as the output of the collective behavior of multicellular systems. The mathematical theory, developed at a well defined observation and representation scale, needs to be consistent with the whole set of scales which represent the system.

The analysis of this issue, with reference to the greater scale, generates, quite naturally, the following questions:

i) Which type of macroscopic phenomena can be accurately described by models at the multicellular scale?

ii) Supposing that the above problems are technically solved, is it sufficient to describe the overall system, or, is it necessary to consider the problem as composed of a series of interacting sub-systems, each operating at a specific scale?

iii) Is the selection of one scale only sufficient to model the behavior of each subsystem, or, even at this level, it is necessary to consider more than one scale?

Several authors have proposed various models of the macroscopic behavior of cancer tissues. A survey of models is delivered in the review paper by Bellomo et al. (2003), bearing in mind that it is necessary dealing with moving boundary problems, among others, Bertuzzi et al. (2004), (2006), Cui and Friedman (2003), Friedman and Lolas (2005), Tao et al. (2004), (2007). In some cases different scales are taken into account in the model, e.g Chaplain and Lolas (2005), Levine et al. (2001), Owen and Sherratt (2000), Bru et al. (1998), (2005). Finally various models have been recently proposed to describe networks of interconnected systems of several interacting subsystems, see for example, Alarcon et al. (2004), Byrne et al. (2006), Anderson (2005).

However complex is the multiscale problem, one has to tackle the problem of selecting the structure of mathematical equations to be used. Asymptotic methods can be used to derive macroscopic equations from the underlying microscopic description. Various papers have been recently proposed towards the above aim by presenting models where spatial phenomena are modelled by adding to the spatially homogeneous equations a stochastic perturbation corresponding to a ve-

locity jump process (Othmer and Hillen (2002), Stevens (2002), Lachowicz (2005), Chalub et al. (2006)).

It is worth stressing again that the analysis needs to be implemented by a theoretical input from biology. Specifically, the analysis of Bellomo and Bellouquid (2006) shows how, under a proper scaling parabolic, a diffusion term with source are obtained, where the presence of a source term appears if the proliferation rate is sufficiently large.

Therefore, we simply remark that the structure of the equations of tissues depends again on the predominance of one of the three aspects of the biological dynamics, i.e. encounter rate between cells, mutations, and proliferating/destructive events, with respect to the other two. Moreover, the structure of the mathematical equations modelling tissues may evolve in time due to the afore mentioned dynamics. So far, the formal approach provides a scenario of structurally different macroscopic equations to be identified by a characterization by biological sciences.

6 References

- Alarcón T., Byrne H.M., and Maini P.K., (2004), A mathematical model of the effects of hypoxia on the cell-cycle of normal cancer cells, *J. Theoretical Biology* **229**, 395–411.
- Anderson A.R.A., (2005), A hybrid mathematical model of solid tumor invasion: the importance of cell adesion, *Mathematical Medicine and Biology* **22** 163–186.
- Anderson A.R.A., Weaver A.M., Cummings P.T., and Quaranta V., (2006), Tumor morphology and phenoypic evolution driven by selective pressure from the microenvironment, *Cell* **127**, 905–915.
- Baylin S.B., and Ohm J.E., (2006), Epigenetic gene silencing in cancer - a mechanism for early oncogeninc pathway addition, *Naure Reviews - Cancer* **6**, 107–116.
- Bellomo N., and Forni G., (1994), Dynamics of tumor interaction with the host immune system, *Mathematical Computer Modelling* **20**, 107–122.
- Bellomo N., De Angelis E., and Preziosi L., (2003), Multiscale modeling and mathematical problems related to tumor evolution and medical therapy, *Journal of Theoretical Medicine* **5**, 111-136.
- Bellomo N., and Bellouquid A., (2006), On the onset of nonlinearity for diffusion models of binary mixtures of biological materials by asymptotic analysis, *International J. Nonlinear Mechanics* **41**, 281–293.

- Bellomo N., and Forni G., (2006), Looking for new paradigms towards a biological-mathematical theory of complex multicellular systems, *Mathematical Models and Methods in Applied Sciences* **16**, 1001–1029.
- Bellomo N., and Maini P.K., (2006), Preface, *Mathematical Models and Methods in Applied Sciences*, **16**(7b), iii–vii.
- Bertuzzi A., Fasano A., and Gandolfi A., (2004), A free boundary problem with unilateral constraints describing the evolution of a tumor cord under the influence of cell killing agents, *SIAM J. Math. Anal.* **36**, 882–915.
- Bertuzzi A., Fasano A., and Gandolfi A., (2005), A mathematical model for tumor cords incorporating the flow of interstitial fluids, *Mathematical Models and Methods in Applied Sciences* **15**, 1735–1777.
- Bellouquid A., and Delitala M., (2004), Kinetic (cellular) models of cell progression and competition with the immune system, *Z. Angewandte Mathematical Physics*, **55**, 295–317.
- Bellouquid A., and Delitala M., (2005), Mathematical methods and tools of kinetic theory towards modelling complex biological systems, *Mathematical Models and Methods in Applied Sciences*, **15**, 1639–1666.
- Bellouquid A., and Delitala M., (2006), ***Modelling Complex Multicellular Systems - A Kinetic Theory Approach***, Birkäuser, Boston.
- Blankenstein T., (2005), The role of tumor stroma in the interaction between tumor and immune system, *Current Opinion Immunology* **17**, 180–186.
- Blume-Jensen P., and Hunter T., (2001), Oncogenic kinase signalling, *Nature* **411**, 355–365.
- Brazzoli I., and Chauviere A., (2006), On the discrete kinetic theory for active particles. Modelling the immune competition, *Computational and Mathematical Methods in Medicine* **7**, 142–1158.
- Bru A., Pastor J.M., Feraud I., Bru I., Melle S., and Berenguer C., (1998), Super-rough dynamics on tumor growth, *Physical Review Letters* **81**, 4008–4011.
- Bru A. and Herrero M.A., (2006) From the physical laws of tumor growth to modelling cancer process, *Mathematical Models and Methods in Applied Sciences* **16**, 1199–1218.
- Byrne H.M., Alarcon T.A., Murphy J., and Maini P.K., (2006), Modelling the response of vascular tumours to chemotherapy: a multiscale approach, *Mathematical Models and Methods in Applied Sciences* **16**, 1219–1241.

- Chalub F., Dolak-Struss Y., Markowich P., Oeltz D., Schmeiser C., and Soref A., (2006), Model hierarchies for cell aggregation by chemotaxis, *Mathematical Models and Methods in Applied Sciences* **16**, 1173–1198.
- Chaplain M.A.J., and Lolas G., (2005), Spatio-temporal heterogeneity arising in a mathematical model of cancer invasion of tissue, *Mathematical Models and Methods in Applied Sciences* **15**, 1685–1734.
- Cui S., and Friedman A., (2003), Hyperbolic free boundary problem modelling tumor growth, *Interfaces and Free Boundaries*, **5**, 159–181.
- De Angelis E., and Jabin P.E., (2003), Qualitative analysis of a mean field model of tumor-immune system competition, *Mathematical Models and Methods in Applied Sciences* **13**, 187–206.
- De Angelis E., and Jabin P.E., (2005), Qualitative Analysis of a mean field model of tumor-immune system competition, *Mathematical Methods in Applied Sciences* **28**, 2061–2083.
- Delitala M. and Forni G., (2007), From the mathematical kinetic theory of active particles to modelling genetic mutations and immune competition, Internal Report, Dept. Mathematics, Politecnico, Torino.
- De Pillis L.G., Gu W., and Radunskaya A.E., (2006), Mixed immunotherapy and chemotherapy of tumors: modeling, applications and biological interpretations, *J. Theoretical Biology* **238**, 841–862.
- De Pillis L.G., D.G. Mallet, and Radunskaya, A.E., (2006), Spatial tumor-immune modeling, *Computational and Mathematical methods in Medicine* **7**, 159–176.
- Derbel L., (2004), Analysis of a new model for tumor-immune system competition including long time scale effects, *Mathematical Models and Methods in Applied Sciences* **14**, 1657–1681.
- d’Onofrio A., (2006), Tumor-immune system interaction: modeling the tumor-stimulated proliferation of effectors and immunotherapy, *Mathematical Models and Methods in Applied Sciences* **16**, 1375–1401.
- Dunn G.P., Bruce A.T., Ikeda H., Old L.J., Schreiber R.D., (2002), Cancer immunoeediting: from immunosurveillance to tumor escape, *Nature Immunology* **3**, 991–998.
- Friendl P., de Boer A.T., and Gunzer M., Tuning immune responses: diversity and adaptation of the immunological synapse, *Nature Review Immunology*, **5** (2005) 532–545.

- Gatenby R.A., Vincent T.L., and Gillies R.J., (2005), Evolutionary dynamics in carcinogenesis, *Mathematical Models and Methods in Applied Sciences* **15**, 1619–1638.
- Greller L., Tobin F., and Poste G., (1996), Tumor Heterogeneity and progression: Conceptual foundation for modeling, *Invasion and Metastasis* **16**, 177–208.
- Friedman A. and Lolas G., (2005), Analysis of a mathematical model of tumor lymphangiogenesis, *Mathematical Models and Methods in Applied Sciences* **15**, 95–107.
- Hanahan D., and Weinberg R.A., (2000), The Hallmarks of cancer, *Cell* **100**, 57–70.
- Hartwell H.L., Hopfield J.J., Leibner S., and Murray A.W., (1999), From molecular to modular cell biology, *Nature* **402**, c47-c52.
- Kolev, (2003), Mathematical modeling of the competition between acquired immunity and cancer, *Applied Mathematics Computer Science* **13**, 289–297.
- Kolev M., (2005), A mathematical model of cellular immune response to leukemia, *Mathematical Computer Modelling* **41**, 1071–1082.
- Kolev M., Kozłowska E., and Lachowicz M., (2005), Mathematical model of tumor invasion along linear or tubular structures, *Mathematical Computer Modelling* **41**, 1083–1096.
- Komarova N., Stochastic modeling of drug resistance in cancer, *J. Theoret. Biol.*, **239** (2006) 351–366.
- Lachowicz M., (2005), Micro and meso scales of description corresponding to a model of tissue invasion by solid tumors, *Mathematical Models and Methods in Applied Sciences* **15**, 1667–1684.
- Lollini P.L., Motta S., and Pappalardo F., (2006), Modelling tumor immunology, *Mathematical Models and Methods in Applied Sciences* **16**, 1091–1125.
- Levine H., Pamuk S., Sleeman B., and Nilsen-Hamilton S., (2001), Mathematical modeling of capillary formation and development in tumor angiogenesis: Penetration into the stroma, *Bulletin Mathematical Biology* **63**, 801–863.
- May R.M., (2004), Uses and abuses of mathematics in biology, *Science* **303**, 790–793.
- Merlo L.M.F., Pepper J.W., Reid B.J., Maley C.C., (2006), Cancer as an evolutionary and ecological process, *Nature Reviews - Cancer* **6**, 924–935.

- Novak M.A., and Sigmund K., (2004), Evolutionary dynamics of biological games, *Science* **303**, 793–799.
- Nowell P.C., (2002), Tumor progression: a brief historical perspective, *Seminars in Cancer Biology* **12**, 261–266.
- Othmer H.G. and Hillen T., (2002), The diffusion limit of transport equations II: chemotaxis equations, *SIAM J. Applied Mathematics* **62**, 1222–1250.
- Owen T. and Sherratt J., (2000), Pattern formation and spatio-temporal irregularity in a model for macrophage-tumor interactions, *J. Theoretical Biology* **189**, 63-80.
- Pappalardo F., Lollini P.L., Castiglione F., and Motta S., (2005), Modelling and simulation of cancer immunoprevention vaccine, *Bioinformatics* **21**, 2891–2897.
- Reed R., (2004), Why is mathematical biology so hard?, *Notices of the American Mathematical Society* **51**, 338–342.
- Spencer S.L., Berryman M.J., Garcia J.A., and Abbott D., (2004), An ordinary differential equation model for multistep transformation to cancer, *J. Theoretical Biology* **231**, 515-524.
- Stevens A., (2002), The derivation of chemotaxis equations as limit dynamics of moderately interacting stochastic many-particles systems, *SIAM J. Applied Mathematics* **61**, 183-212.
- Tao Y., Yoshida N., and Guo Q., (2004), Nonlinear analysis of a model of vascular tumor growth and treatment, *Nonlinearity* **17**, 867–895.
- Tao Y. and Zhang H., (2007), A parabolic-hyperbolic free boundary problem modelling tumor treatment with virus, *Mathematical Models and Methods Applied Sciences* **17**, 63–80.