Population ecology of heterotypic tumour cell cultures

M. Sega and R. Chignola

Department of Biotechnology, University of Verona, I-37134, Verona, Italy

Received 8 May 2014; revision accepted 20 June 2014

Abstract

Objectives: Here, we propose a population ecology perspective to describe dynamic interplay between human leukaemia and cervical cancer cells growing together in the same environment.

Materials and methods: MOLT-3 (human T-lymphoblastic leukaemia) and HeLa (human cervical adenocarcinoma) cells were grown together or alone. Living cells were measured using flow cytometry, by counting propidium iodide-negative cells either CD5⁺ (MOLT-3) or CD55⁺ (HeLa). We developed a mathematical model to take into account possible interactions between cells and among cells and their environmental niches. Model equations were then fitted to growth data.

Results: Ecological interactions that require direct cell contact and indirect mechanisms acting on cell niches, successfully modelled cell population growth. Predicted heterotypic adhesion between the two different cell types was demonstrated experimentally.

Conclusions: Theoretical ecology can be assayed using human cells and, most importantly, it can provide a conceptual framework to describe and understand evolution of mixed tumour cell populations.

Introduction

Cells in a tissue behave in a similar way to animal populations in an ecosystem: they co-operate, compete or simply coexist with other cell populations; they consume, provide and exchange resources and interact with the microenvironment and thus indirectly also with the other cells. In addition akin to animal populations, cells can evolve, undergo Darwinian processes of selection,

co-operate or compete with other cells of the same type or those of different histological origin. All these processes have been well documented and have important implications for many aspects of animal physiology and pathology, for example, tissue maintenance and homeostasis, angiogenesis, immune responses, growth and spreading of tumour cells [(1,2) and references cited therein].

These considerations support the notion that cells and their environmental niches constitute a cellular ecosystem and that this paradigm describes behaviour and fate of animal cells. Mathematical models originally developed in the field of population ecology have been used to predict behaviour of cell populations, particularly in the context of tumour biology (3–5). Indeed, complex interplay between cells and their niches has been modelled using a stochastic approach, the model of which successfully describing growth of experimental solid tumours (6). To the best of our knowledge only a few attempts have been made to connect model predictions to experimental data. This is a key point, as it is only by fitting experimental data to model equations that values of parameters can be estimated and range of validity of mathematical models determined.

We propose to start accomplishing this task using neat *in vitro* experimental setups. As proof-of-concept, we have investigated dynamic interactions of two different cell populations growing together. It is becoming more and more clear that fate of individual cancers depends upon complex interactions between cells that belong to different populations, but occupy the same environment (1,2); the challenge in modern oncology is to uncover such hidden and untidy interplay. For example, thymic epithelial cells promote survival of human T-cell acute lymphoblastic leukaemia blasts (T-ALL) by furnishing appropriate signals such as interleukin-7 (7). Normal tissue-derived epithelial cells can exert protective effects on survival of adult T-cell leukaemia/lymphoma and these effects partially account for high resistance to anti-leukaemic therapies in patients (8). On the other hand, transformed epithelial cells are also known to interact and affect functions of normal blood cells. For example, HeLa

Correspondence: R. Chignola, Department of Biotechnology, University of Verona, Strada Le Grazie 15 – CV1, I-37134 Verona, Italy. Tel.: +39 045 8027953; Fax: +39 045 8027925; E-mail: roberto.chignola@univr.it

cells have been observed to inhibit IL-2-mediated proliferation of human peripheral blood mononuclear cells, a possible mechanism of tumour escape from surveillance of the immune system (9). To complete the picture, tumour epithelial cells and T-leukaemia cells have recently been shown to interact and exchange information. HeLa cells can inhibit human T-cell leukaemia type 1 virus (HTLV-1) expression when co-cultured with cells drawn from T-ALL patients (10). Thus interplay between different cell populations can indeed determine the fate of cancers.

The studies cited above demonstrate that proliferating epithelial cells and T lymphocytes mutually interact and suggest that they can be used to investigate dynamic interplay between different cell types. For this reason, we chose the well-characterized MOLT-3 and HeLa human cell lines to set up an experimental model of a mixed cell ecosystem.

Materials and methods

Cells and growth assays

Cells from human T-lymphoblastic line MOLT-3 (ATCC number CRL-1552) and from human cervical carcinoma line HeLa (ATCC number CCL-2) were cultured at 37 °C in a humidified 5% CO₂ atmosphere, in RPMI 1640 (Biocrom AG, Berlin, Germany) supplemented with 2 mM glutamine (Sigma, Milan, Italy), 35 mg/l gentamycin (Biocrom AG) and 10% heat-inactivated foetal bovine serum (Biocrom AG). Co-culture experiments were carried out by plating cells into wells of 24-well culture plates at 4:1 HeLa:MOLT-3 ratio, total cell density 2000 cell/ml (final volume 2.5 ml). 4:1 cell ratio was chosen on the basis of preliminary cell population growth experiments with both cell lines. MOLT-3 cells grew faster than HeLa cells, and 4:1 plating ratio assured that MOLT-3 did not significantly outnumber HeLa during co-culture experiments (Fig. 2). Individual HeLa and MOLT-3 cell cultures were also grown in parallel by plating cells in 24-well culture plates at the same initial cell density and same absolute cell counts used in co-culture experiments. Cells were collected at different time points, analysed and counted by flow cytometry. For statistical purposes, three independent MOLT-3, HeLa and MOLT-3 + HeLa cultures were cultured and analysed, and data were finally expressed as mean $\pm$ SD. To test reproducibility of the method, experiments were also repeated by two operators at different times and using different cell batches.

Flow cytometry

Cells were harvested by treatment with 0.5 ml of 10 mM EDTA in PBS at 37 °C for 15 min. After washings in

complete RPMI, samples were incubated for 30 min at 4 °C in the dark with specific antibodies: anti-human CD5, R-PE conjugated, clone DK23 (DakoCytomation, Milan, Italy) for MOLT-3 cells and anti-human CD55 FITC (eBioscience, San Diego, CA, U.S.A.) for HeLa cells. Cells were then stained with propidium iodide staining solution (eBioscience), and analysed using a Guava easyCyte™ 5 flow cytometer, controlled by GuavaSoft v.2.7 software (Merck Millipore, Billerica, MA, U.S.A.). This instrument is equipped with a micro-capillary system that allows direct sampling of given amounts of sample volume by aspiration, thus eliminating the need for sheath fluid. A positive displacement syringe pump enables sampling and tunes flow rates. This cytometer thus performs absolute cell counts in measured amounts of sample volume without the need of reference beads, and returns these parameters to the operator.

The cytometer was equipped with 488 nm, 20 mW, blue laser light, and forward scatter (FSC) photodiode and side scatter (SSC) photomultiplier. Green fluorescence 525/30 filter, yellow 583/26 and red 680/30 filters allow analysis of fluorescence emissions from samples. Calibration of the cytometer was routinely checked using the Guava EasyCheck kit (Merck Millipore, Billerica, MA, U.S.A.) according to the manufacturer's instructions. The kit is composed of known concentration of fluorescent particles. Also, scattering properties and fluorescence emission of particles in the three channels were known. Counting accuracy was always $\pm 10\%$ and both counting precision and fluorescence detection was $<10\%$. Data were collected by setting the following gain controls and fluorescence compensations: threshold level on channel 123, parameter FSC; gain level 3.08, par. FSC; gain level 3.36, par. SSC; gain level 3.08, par. green fluorescence; gain level 3.08, par. yellow fluorescence; gain 17.4, par. red fluorescence; compensations: yellow – 8.3% green, red – 3.2% green, yellow – 6.0% red.

Heterotypic cell adhesion

Adhesion between MOLT-3 and HeLa cells was demonstrated by allowing HeLa cells to adhere to the surface of a glass capillary tube open at both extremities. Internal diameter of the tube was 2 mm, length 90 mm. By assuming that cell radius is $\sim 5 \mu\text{m}$, we estimated that $\sim 7 \times 10^6$ HeLa cells were sufficient to layer over the entire surface of the tube. We injected an excess of 9×10^6 cells into the capillary and they were allowed to adhere overnight at 37 °C in a 5% CO₂ atmosphere. The capillary was kept under gentle rotation along its major axis to expose the whole surface to cell adhesion. The capillary was then connected to a peristaltic pump and extensively flushed with RPMI to remove unbound

cells. Calibration experiments showed that under these conditions a volume of 8 ml medium flushed at the rate of 1.6 ml/min was sufficient to remove 100% unbound cells. MOLT-3 cells (1:1, HeLa:MOLT-3) were then injected into the capillary tube and the system was incubated overnight at 37 °C and 5% CO₂ under gentle rotation. Excess of 12 ml medium was flushed at 1.6 ml/min and this fraction, which contained unbound MOLT-3 cells, was discarded. Rate was then progressively increased and fractions of 2.5 ml volume collected.

Cells in each fraction were quantified by measuring their ATP content. ATP determinations were carried out using luciferin/luciferase method using CellTiter-Glo Luminescent Cell Viability Assay kit (Promega, Milan, Italy). Emitted light was measured using a microplate luminometer (FL_X 800, Bio-Tek Instruments, Winooski, VT, U.S.A.).

Modelling interplay between cells

We extended the nutrient-depletion (ND) model developed by Tracqui *et al.* (11) that describes population expansion of cells in a closed environment, such as a culture flask. In its basic formulation, and using the same notation as in (11), the dimensionless model is defined by the following set of differential equations:

$$\begin{aligned}\frac{dn(t)}{dt} &= \beta n(t)q(t) - kn(t)(1 - q(t)) \\ \frac{dq(t)}{dt} &= -\alpha n(t)q(t) \\ n(t_0) &= q(t_0) = 1\end{aligned}\quad (1)$$

where $n(t)$ is cell density, $q(t)$ nutrient content of the environment, and α , β and k are positive parameters. According to this model, exponential proliferation of the cells is limited by nutrient availability $q(t)$ in the environment and eventually the cell population collapses when nutrients have all been consumed. The variable $q(t)$, therefore, models time evolution of the cellular niche.

For a general cell population, i that interacts with a population j the equations become:

$$\begin{aligned}\frac{dn_i(t)}{dt} &= \beta n_i(t)q_i(t) - kn_i(t)(1 - q_i(t)) + f(n_i, n_j, t) \\ \frac{dq_i(t)}{dt} &= -\alpha n_i(t)q_i(t) + g(n_j, q_i, q_j, t)\end{aligned}\quad (2)$$

where functions f and g describe possible interactions between cells and among cells and niches respectively. The equations were solved numerically and were fitted to experimental data. Goodness of fits was determined by calculating reduced chi-squared $\chi^2_{\text{red}} = \chi^2/\text{df}$, where

df is the degrees of freedom. All calculations were carried out using Mathematica software (v. 9.0.1.0; Wolfram Research Inc., Champaign, IL, U.S.A.).

Results

Experimental data

Flow cytometry allowed us to measure cell population growth kinetics of HeLa and MOLT-3 cell populations no matter whether they were grown alone or cultured together (Fig. 1). To test reproducibility of growth assays, data were collected in two independent experiments carried out by different operators with different cell batches (Fig. 2). Initial exponential growth phases of both cell lines did not differ significantly if cells were co-cultured or grown alone. Later, however, when cultured together, growth kinetics of both cell lines deviated from behaviour shown by isolated cultures. MOLT-3 cells appeared to be at an advantage by growing with HeLa cells and density of population peaked at higher values with respect to MOLT-3 cell population grown alone. Conversely, HeLa cells appeared disadvantaged and when co-cultured with MOLT-3 cells their density peaked at lower values than those shown by isolated HeLa cell populations. At later time points, in co-culture experiments, density of the HeLa population started to decay earlier than that of isolated cell populations. This part of the growth kinetics was dominated by nutrient consumption and accumulation of waste toxic factors in the environment, and thus by cell death. More generally, it reflects exhaustion of the cellular niche that progressively lost capacity to sustain cell life (11). Importantly, peak at higher cell densities observed for MOLT-3 cells in co-culture experiments, peak at lower cell densities, as well as faster decay rates shown by HeLa cell populations in co-culture experiments, were well reproduced in independent assays.

Taken together, the data suggest that interactions between MOLT-3 and HeLa cells were complex and involved both cell populations and their niches.

Theoretical interpretation of experimental data

We assume that each cell population and its own niche formed an individual cell ecosystem (Fig. 3). In co-culture experiments, at increasing cell densities, the two cell ecosystems might interact, and such interactions might affect molecular composition of niches and/or directly the cell populations (Fig. 3). This assumption was supported by the observation that at the beginning of growth assays, when both cell populations were

Figure 1. Representative raw data from flow cytometry experiment. *Panel A:* distribution of un-gated HeLa cell population in log-FSC versus log-SSC plot. *Panel B:* distribution of un-gated MOLT-3 cell population in log-FSC versus log-SSC plot. *Panel C:* distribution of mixed HeLa and MOLT-3 cell populations (co-culture experiment) in log-FSC versus log-SSC plot. Light scattering properties of cell lines overlap, thus cannot allow discrimination between the two cell populations (compare with Panels A and B). *Panel D:* gated R1 mixed cell population shown in previous panel labelled with fluorescent anti-CD5 and anti-CD55 monoclonal antibodies. MOLT-3 and HeLa cell populations clearly identified. *Panels E and F:* MOLT-3 and HeLa cell populations identified in previous panels separated electronically using indicated gates. In these panels, both cell populations are labelled with specific monoclonal antibody and with propidium iodide (PI) to exclude dead cells from the analysis. Live cells only quantified.

small and their interactions less probable, both cell populations grew at the same rates as isolated populations (Fig. 2).

Interactions of each population with its own niche are described by eqn (1). This model can be fitted to growth data measured for isolated cell cultures to estimate values of parameters α , β and k (Table 1). The assumption discussed above implies that values of these parameters must be considered as fixed in eqn (2) that

contain the functions that model interactions between cell ecosystems in co-culture experiments. In the absence of interactions between the two cell ecosystems, each cell population would behave as an independent ecological system, that is, as an isolated culture. In eqn (2), functions f and g must be defined. A list of possible functions is given in Table 2. We found that the following model best fitted the experimental data:

Figure 2. Growth of isolated and mixed cell populations. Rows show data collected in two independent experiments carried out by different operators with different cell batches, whereas columns show data corresponding to MOLT-3 and HeLa cells as indicated. In all panels, data measured for isolated cell cultures are represented by open symbols whereas those from the co-cultures by closed symbols. Data expressed as cell density normalized with respect to initial value (initial cell density set = 1).

Figure 3. Modelling interactions between cell ecosystems. Two generic cell populations n_1 and n_2 and their environmental niches q_1 and q_2 (dashed areas) form two cell ecosystems. Interactions between the two ecosystems (for simplicity, the figure shows only one-way interactions of one ecosystem with the other) can be indirect (1. and 2.) or direct (3.) In 1, the population modifies a molecular component (by means, e.g. of enzymatic reaction) that is present in its niche and product diffuses to the other niche, modifies its composition and thus affects growth of the second population. In 2, the first population secretes a molecule that diffuses to the other niche, modifies its composition and affects growth of the other cell population. In 3, members of one population can directly affect growth of the members of the other population. Processes may include triggering of intracellular signalling cascade by receptors through secretion of specific ligands and/or by physical contact to adhesion molecules and receptors.

$$\begin{aligned}\frac{dn_1(t)}{dt} &= \beta_1 n_1(t) q_1(t) - k_1 n_1(t) (1 - q_1(t)) + \gamma_1 n_1(t) n_2(t) \\ \frac{dq_1(t)}{dt} &= -\alpha_1 n_1(t) q_1(t)\end{aligned}$$

$$\begin{aligned}\frac{dn_2(t)}{dt} &= \beta_2 n_2(t) q_2(t) - k_2 n_2(t) (1 - q_2(t)) - \gamma_2 n_1(t) n_2(t) \\ \frac{dq_2(t)}{dt} &= -\alpha_2 n_2(t) q_2(t) - \epsilon_2 n_1(t)\end{aligned}\quad (3)$$

where indices 1 and 2 identify MOLT-3 and HeLa cells respectively. Estimated parameter values are given in Table 1 and an example of the fits is shown in Fig. 4.

In Table 1, it can be observed that fits of the two independent data sets provide parameter estimates that do not differ significantly (Student's t -test, 5% probability threshold) except for values of parameter k estimated for isolated MOLT-3 cultures. This difference reflects different decay of the two cell cultures that can be appreciated in Fig. 2, and that pertains to biological variability. In terms of model equations, this effect is interpreted in terms of different sensitivity to nutrient depletion of the two cell cultures, and hence of different death rates. Importantly, however, interactions between MOLT-3 and HeLa cells were not affected by this difference (compare values of parameters γ and ϵ estimated for the two experiments in Table 1). This further supports the initial assumption that a cell population and its own niche form an individual cell ecosystem.

Table 1. Estimated parameter values and goodness-of-fit statistics^a

Cells	α	β	k	$\chi^2_{\text{red}(1)}$	γ	ε	$\chi^2_{\text{red}(3)}$
MOLT-3	$3.0 \pm 1.3 \times 10^{-4}$	0.81 ± 0.02	0.99 ± 0.5	1.15	$2.7 \pm 0.7 \times 10^{-4}$		2.3
HeLa	$4.1 \pm 0.7 \times 10^{-4}$	0.86 ± 0.02	0.30 ± 0.05	1.97	$3.8 \pm 1.8 \times 10^{-4}$	$3.7 \pm 1.2 \times 10^{-4}$	
MOLT-3	$1.5 \pm 0.1 \times 10^{-4}$	0.88 ± 0.04	2.6 ± 0.04	3.40	$4.2 \pm 0.3 \times 10^{-4}$		1.01
HeLa	$8.6 \pm 1.3 \times 10^{-4}$	0.96 ± 0.05	0.2 ± 0.04	1.02	$1.7 \pm 0.7 \times 10^{-4}$	$2.3 \pm 0.4 \times 10^{-4}$	

^aParameters are all defined, e.g. in eqn (3) where indices 1 and 2 refer to MOLT-3 and HeLa cells respectively. Two rows separated by line show estimates obtained by fit of two independent data sets. Parameters α , β and k were estimated by fitting eqn (1) to growth data of individually cultured MOLT-3 and HeLa cells. Goodness of both fits is indicated ($\chi^2_{\text{red}(1)}$). Parameters γ and ε were estimated by simultaneous fitting of growth data measured for MOLT-3 and HeLa cell co-cultures with eqn 3. Estimated values of parameters α , β and k , in this case, were considered constant and equal to values estimated for growth of individual cell populations (see in the text). Goodness of simultaneous fit is indicated in the final column ($\chi^2_{\text{red}(3)}$).

Table 2. List of possible interactions between cell ecosystems^a

Functions	Interpretation
$f(n_i, n_j, t) \propto$	
$+n_i n_j$	prey-predation-like: predation of preys n_j
$-n_i n_j$	prey-predation-like: predation by predators n_j
$+n_j$	direct stimulation
$-n_j$	direct inhibition
$g(n_j, q_i, q_j t) \propto$	
$+n_j q_i$	niche regeneration through the activity of the population n_j on molecules of the niche itself
$-n_j q_i$	niche consumption by the population n_j
$+n_j$	niche regeneration through secretion of molecular factors
$-n_j$	niche intoxication
$+n_j q_j$	niche regeneration through the activity of the population n_j on molecules of its own niche
$-n_j q_j$	niche intoxication through the activity of the population n_j on molecules of its own niche

^aAssumed that these interactions concern cell population n_i and its niche q_i .

Figure 4. Fit of growth data (see experiment 1, Fig. 2) with model equations. Data expressed as cell density normalized with respect to initial value (initial cell density set = 1). In both panels, data measured for isolated cell cultures are represented by open symbols whereas those from the co-cultures by closed symbols. Lines represent best fitting of experimental data with eqn (1) (isolated cultures) or with eqn (3) (co-culture experiments) as explained in the text.

Partial validation of the model

Equation (3) describe both direct interactions between the two cell populations and indirect interactions through modification of a population niche by the other cell population. The possible indirect interactions are difficult to measure and to dissect out as underlying molecular mechanisms probably act in the cell microenvironment and require simultaneous presence of both

cell populations in close proximity, to occur. However, predation-like terms $\gamma_{ij} n_i(t) n_j(t)$ in eqn (3) suggest that MOLT-3 and HeLa cells somehow interacted directly.

Cell adhesion was investigated using a capillary flow experiment (Materials and methods section). Data expressed in ATP luminescence arbitrary units normalized per volume of sampled fraction are shown in Fig. 5. These clearly show that MOLT-3 cells and HeLa cells adhered quite strongly. High shear stress was

Figure 5. Adhesion between MOLT-3 and HeLa cells. Cell adhesion was investigated in a capillary flow system, flux rate proportional to shear stress. Cells detach when shear stress roughly exerts force higher than adhesion force, but the underlying biophysics are complicated by mechanisms such as cell rolling on to the adhesive layer (20). Adhesion of MOLT-3 cells to HeLa cells (circles). For comparison purposes, we carried out the same experiment with Caco-2 (ATCC HTB-37) cells (squares) and CFPAC-1 (ATCC CRL-1918) cells (triangles). These are epithelial cell lines derived from a human colorectal adenocarcinoma and a human ductal pancreatic adenocarcinoma respectively. Adhesion of MOLT-3 cells to Caco-2 and CFPAC-1 is much less intense than that shown by MOLT-3 to HeLa cells.

required to detach MOLT-3 cells from HeLa, higher than that shown by the same cells when they interact with Caco-2 or CFPAC-1 cells. It is worth noting that HeLa, Caco-2 and CFPAC-1 are all epithelial-like cells.

Cell adhesion between MOLT-3 and HeLa cells was also observed by light microscopy (data not shown).

Discussion

Concepts developed in the field of population ecology have already been used to describe life and fate of cells in body tissues (3–5,12–15). Thus we have been able to proceed a step further and exploit quantitative methods of population ecology to investigate behaviour of cells to understand such dynamics and the basis of tissue physiology and pathology.

Such attempts have been made in the past; for example, oscillatory dynamics of different lymphocyte populations observed during an immune response have been interpreted to be the result of predation-prey-like behaviour acting between different cell populations (16,17). However, both biological aspects and theoretical models were too complex at that time, and could not be managed to solve experimental approaches until computerized technologies had been able to obtain firm conclusions.

As proof-of-concept, we studied a simple experimental ecosystem and here have shown that its behaviour can

be qualitatively and quantitatively described using models inspired by population ecology. Relevance of our findings is 2-fold. On the one hand, this approach can allow understanding of all possible, relevant interactions between cells and their niches and thus suggest which mechanisms are at work in a given biological process at the basis of tissue physio-pathology. The predicted, and then experimentally verified, adhesion between MOLT-3 and HeLa cells is one example. In the field of experimental oncology, this possibility might be exploited to investigate dynamic cross-talk between tumour cells and cells from surrounding tissues. Among these, stromal cells and endothelial cells are well known to play a fundamental role in determining evolution and fate of tumours (1,2 and references cited therein). Also sub-populations of immune cells exist that can outcompete tumour cells or paradoxically co-operate with them and favour tumour invasion, growth and metastasis (18). Unravelling dynamics that underly intimate and intricate cross-talk between tumour cells and other cell types will allow us to improve our knowledge of the disease and perhaps explain some key cancer properties such as tumour heterogeneity, or suggest new therapeutic approaches with normal cells engineered to compete with tumour cells.

On the other hand, our findings show that it is possible to use cell cultures to test validity of theoretical models from population ecology. Here, we have aimed to prove this concept using new experimental and theoretical approaches, and thus mainly focused our efforts on both data reproducibility and model validation. Interesting questions have been left open, however. For example, sensitivity of the experimental set-up to different initial conditions (such as initial cell density ratios) or to continuous medium exchange, as in a bioreactor, that would open up the system and possibly allow the onset of oscillatory cell behaviour (11,19).

It is possible that cells might be engineered to express new phenotypes and behaviours to reproduce at will a desired microscopic ecosystem.

Acknowledgements

We thank Olivia Marini, Luca Ligabò, Alberta Giovanazzi and Giulia Fracca, former students of the Biotechnology course of the University of Verona. Under our supervision, they enthusiastically carried out part of the experimental study reported in this article.

References

- 1 Hanahan D, Weinberg RA (2000) Hallmarks of cancer. *Cell* **100**, 57–70.
- 2 Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* **144**, 646–674.

- 3 Gatenby RA (1991) Population ecology issues in tumor growth. *Cancer Res.* **51**, 2541–2547.
- 4 Adam JA, Bellomo N (1996) A Survey of Models for Tumor-immune System Dynamics. Boston, MA: Birkhäuser, 344 pp.
- 5 Gatenby RA, Vincent TL (2003) Application of quantitative models from population biology and evolutionary game theory to tumor therapeutic strategies. *Mol. Cancer Ther.* **2**, 919–927.
- 6 Chignola R, Dai Pra P, Morato LM, Siri P (2006) Proliferation and death in a binary environment: a stochastic model of cellular ecosystems. *Bull. Math. Biol.* **68**, 1661–1680.
- 7 Scupoli MT, Vinante F, Krampera M, Vincenzi C, Nadali G, Zampieri F *et al.* (2003) Thymic epithelial cells promote survival of human T-cell acute lymphoblastic leukemia blasts: the role of interleukin-7. *Haematologica* **88**, 1229–1237.
- 8 Miyatake Y, Oliveira AL, Jarbouli MA, Ota S, Tomaru U, Teshima T *et al.* (2013) Protective roles of epithelial cells in the survival of adult T-cell leukemia/lymphoma cells. *Am. J. Pathol.* **182**, 1832–1842.
- 9 Logan GJ, Smyth CM, Earl JW, Zaikina I, Rowe PB, Smythe JA *et al.* (2002) HeLa cells cocultured with peripheral blood lymphocytes acquire an immuno-inhibitory phenotype through up-regulation of indoleamine 2,3-dioxygenase activity. *Immunology* **105**, 478–487.
- 10 Kinpara S, Hasegawa A, Utsonomiya A, Nishitsuji H, Furukawa H, Masuda T *et al.* (2009) Stromal cell-mediated suppression of human T-cell leukemia virus type 1 expression in vitro and in vivo by type I interferon. *J. Virol.* **83**, 5101–5108.
- 11 Tracqui P, Liu JW, Collin O, Clement-Lacroix J, Planus E (2005) Global analysis of endothelial cell line proliferation patterns based on nutrient-depletion models: implications for a standardization of cell proliferation assays. *Cell Prolif.* **38**, 119–135.
- 12 Murray JD (2002) Mathematical Biology, 3rd edn. New York: Springer-Verlag, 553 pp.
- 13 McLean AR, Rosado MM, Agnes F, Vasconcellos R, Freitas AA (1997) Resource competition as a mechanism for B cell homeostasis. *Proc. Natl. Acad. Sci. USA* **94**, 5792–5797.
- 14 De Boer RJ, Perelson AS (1998) Target cell limited immune control models of HIV infection: a comparison. *J. Theor. Biol.* **190**, 201–214.
- 15 Komarova NL (2005) Mathematical modelling of tumorigenesis: mission possible. *Curr. Opin. Oncol.* **17**, 39–43.
- 16 Zöller M, Lopatta D, Benato B, Andrichetto G (1985) Oscillation of antibody production and regulatory T cells in response to antigenic stimulation. *Eur. J. Immunol.* **15**, 1198–1203.
- 17 Andrichetto GC, Benato B, Tridente G (1983). Systemic network regulation hypothesis: some experimental evidences. In: Fabris N, Garaci E, Hadden J, Mitchison NA, eds. Immunoregulation, pp. 69–92. New York and London: Plenum Press.
- 18 Talmadge JE, Donkor M, Scholar E (2007) Inflammatory cell infiltration of tumors: Jekyll or Hyde. *Cancer Metastasis Rev.* **26**, 373–400.
- 19 Goldbeter A (1996) Biochemical Oscillations and Cellular Rhythms. Cambridge, UK: Cambridge University Press, 605 pp.
- 20 Garrivier D, Décavé E, Bréchet Y, Bruckert F, Fourcade B (2002) Peeling model for cell detachment. *Eur. Phys. J. E* **8**, 79–97.