LMD konf. darbai, 261–267
1997, Vilnius

Statistical analysis and mathematical modeling of primary and secondary tumors growth in "concomitant immunity" model

R. Eidukevičius (VU), D. Characiejus (LOC)

INTRODUCTION

The phenomenon studied in this paper is called concomitant tumor immunity and is usually defined as inhibition of growth of the secondary tumor during progressive growth of the primary tumor [1]. This indicates the existence of growth regulating mechanisms which are not yet understood. It is well known that T cell-mediated immunity is generated during tumor growth in several systems [2, 3]. In the DBA/2 mouse SL2 tumor system, rejection or inhibition of growth of the secondary tumor occurs in the presence of the primary tumor [2, 4]. The existence of T-lymphocyte-mediated antitumor mechanisms has also been described in this DBA/2-SL2 system [2, 5]. But concomitant immunity is also observed in non-immunogenic tumor models [6, 7] and this fact has been used as an argument against the involvement of immune mechanisms in this phenomenon. The "integrated organ" hypothesis has been proposed, suggesting the existence of organ-specific growth control substances to explain concomitant tumor immunity [8].

To understand better the phenomenon of concomitant tumor immunity we analyzed more carefully the kinetics of growth of the primary and secondary tumors and studied the effect of X-irradiation (which suppress immune system).

MATERIALS AND METHODS

Mice and tumors. Female DBA/2 mice at the age of 8–12 weeks and SL2, a spontaneously arisen, DBA/2-derived lymphoma were used. SL2 cells were maintained by weekly intraperitoneal passage in DBA/2 mice.

Concomitant tumor immunity model. The primary tumors were induced by subcutaneous injection of 10^7 SL2 cells on the left side of the chest into naive mice on day 0, and the secondary tumors were induced by subcutaneous injection of 10^7 SL2 cells on the right side of the chest on day 2. Due to the relatively short time interval between induction of primary and secondary tumors and due to relatively high numbers of tumor cells used in this model, both tumors (primary and secondary) reached measurable volumes by day 5 and could be further measured simultaneously until day 13, i.e. during the period of expression of concomitant tumor immunity in this DBA/2-SL2 system.

Perpendicular diameters (length l and breadth b) of tumors were measured every other day starting from day 5 using a caliper connected to a Digimatic data logger DL-10 (Mitutoyo, Japan). The data were transmitted to a computer and volumes of primary and secondary tumors were calculated as $\frac{1}{6}\pi l b^2$ (the volume of an ellipsoid body).

The specific growth rates of tumors were estimated in the following way. The specific growth rate of a tumor is the growth rate of a unit tumor volume. In other words, the specific growth rate is the increase in tumor volume $V_{t_{i+1}} - V_{t_i}$ between 2 successive tumor volume measurements at t_{t+1} and t_i, divided by the mean tumor volume between those measurements $\frac{V_{t_{i+1}}+V_{t_i}}{2}$ per unit time $t_{i+1} - t_i$. Thus, the specific growth rate of a tumor was estimated as

$$\frac{V_{t_{i+1}} - V_{t_i}}{\frac{V_{t_{i+1}}+V_{t_i}}{2}} \times \frac{1}{t_{i+1} - t_i}.$$

Irradiation. To suppress the lymphoid system, mice of one group were exposed to 4 Gy total body X-irradiation two days prior to implantation of primary tumors (i.e. on day -2).

Mathematical analysis. The Wilcoxon signed-rank test was used for location of one random sample or two related samples. To compare data from two independent samples the two-sample Wilcoxon test was used. The strength of the linear relationship between two variables was measured by the Pearson product-moment correlation. A p-value of 0.05 or less was considered as statistically significant. To perform statistical analysis program packages SAS and Excel were used. A program MAPLE with methods of computer algebra and numeric computations was very useful in analysis of systems of ordinary differential equations.

RESULTS

1. Analysis of tumor volumes

It is well known that the growth of secondary tumor in DBA/2 mouse is completly inhibited when the time interval between implantations is greater or equal to 7 days. It was interesting to see whether the interval of 2 days between implantations in our model was sufficient to cause inhibition of growth of the secondary tumor compared to the primary one. Growth curves of both tumor volumes of control mice group are shown in Fig. 1.

To facilitate comparison of growth rates of primary and secondary tumors we plotted their volumes against age of tumors. Volumes of the tumors would be similar at the same age if tumors grew independently of each other. Fig. 1 shows, however, that the volumes of primary tumors are significantly greater than volumes of secondary tumors at the same age.

Thus, the difference of 2 days of tumor ages is sufficient to induce concomotant tumor immunity. In contrast to these results the primary and the secondary tumors

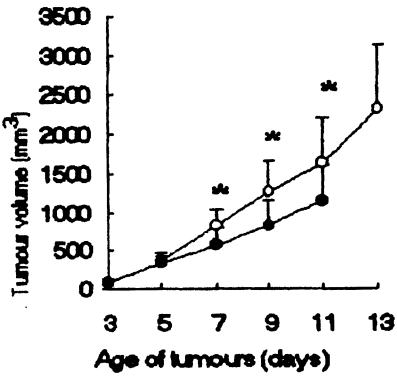

Figure 1. Concomitant tumor immunity when secondary SL2 tumor is implanted in DBA/2 mice two days after the primary SL2 tumor. Primary tumors were induced by subcutaneous injection of 10^7 SL2 cells on the left side of the chest into naive mice on day 0, and secondary tumors were induced by subcutaneous injection of 10^7 SL2 cells on the right side of the chest. Volumes of primary (○) and secondary (●) tumors are plotted against age of tumors. Values of tumor volumes are given as means ±SD of 20 tumors. Secondary tumors reach significantly smaller volumes compared to volumes of primary tumors at the same age. $^*p < 0.001$, Wilcoxon signed-rank test.

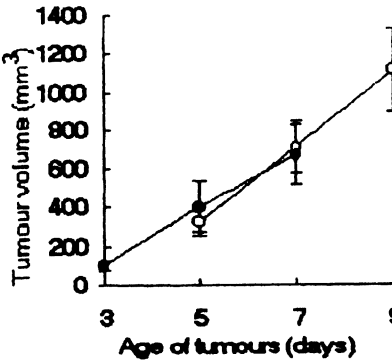

Figure 2. Evidence that concomitant tumor immunity is abrogated by total body X-irradiation with a dose of 4 Gy two days prior to implantation of primary tumors (i.e. on day 2). Volumes of primary (○) and secondary (●) tumors are plotted against age of tumors. The values are given as means ±SD of 20 tumors. The differences between volumes of primary and secondary tumors at the same age are not significant. $p > 0.05$, Wilcoxon signed-rank test.

reach similar volumes at the same tumor age in irridiated mice, i.e. there is no concomitant immunity.

This suggests that lymphoid system is involved in the inhibition of secondary tumor growth.

2. *Analysis of specific growth rates of primary and secondary tumors*

According to definition, specific growth rate is a growth rate per unit of tumor volume. This growth parameter of primary tumor is statistically significantly greater

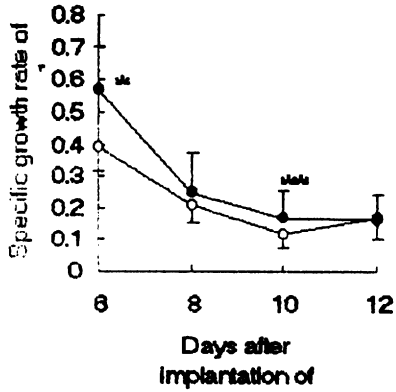

Figure 3. Changes in specific growth rates of primary (○) and secondary (●) tumors. Values of specific growth rates are given as means ±SD of 20 tumors. The specific growth rate of the secondary tumors is significantly higher compared to the primary tumors on days 6 and 10. $*p < 0.001$; $**p < 0.05$. Wilcoxon signed-rank test. The specific growth rate of primary tumors is significantly higher on day 12 than on day 10; $p < 0.05$, Wilcoxon signed-rank test.

than this rate of secondary tumor at day 6, later curves of both specific growth rates decrease, intersect and finally the curve of the first tumor even increases.

It is important that in addition to the process of convergence of specific growth rates the correlation between them also increases from not significant to a strongly significant on 12 day and the linear regression equation is $y = 0.94x + 0.1$.

According to the "integrated organ" hypothesis this synchronization of specific growth rates of both tumors is caused by circulating substances produced by cells in both tumors. Therefore, a correlation between combined volumes of tumors and their specific growth rates should exist. But our results show the absence of the correlation, i.e., this is a contraargument to the "integrated organ" hypothesis.

Although our results suggest involvement of the lymphoid system in synchronization of specific growth rates of primary and secondary tumors, it is difficult to explain this growth regulatory mechanism by cytotoxic T-lymphocytes. Primary and secondary tumors in this model are of the same tumor cell line and, consequently, have the same antigenicity. Thus, tumor cell killing per unit tumor volume by cytotoxic T-lymphocytes would become equal in primary and secondary tumors. As a result, specific growth rates of both tumors would decrease with the same rate and synchronization would not occur. Therefore, it can be hypothesized that the lymphoid system in concomitant immunity influences growth fractions or cell cycle parameters in tumors.

The main conclusions of the analysis are:

1. The time interval of 2 days between tumor implantations is sufficient to induce concomitant tumor immunity.

2. Lymphoid system is involved in syncronization of specific growth rates of both tumors.

3. Most probable that the lymphoid system influences growth and/or cell cycle parameters in tumors because the synchronization of specific growth rates of tumors contradicts with the cytotoxic role of T-lymphocytes, i.e., the uniform mallignant cell killing in both tumors.

MATHEMATICAL MODELS

Various mathematical methods are used to describe dynamics of one population or evolution of a system of interacting populations: dynamical systems with discrete or continuous time, deterministic or stochastic. We used systems of ordinary differential equations and their qualitative analysis. Our attempts to model growth of two tumors based on hypothesis of "integrated organ" were not successful. Also classical models of various kinds of interaction between 2 populations (predator and pray, symbiosis, competition) did not fit our experimental results. The most to appropriate is more complicated model [10] with 4 equations for two solid tumors cell

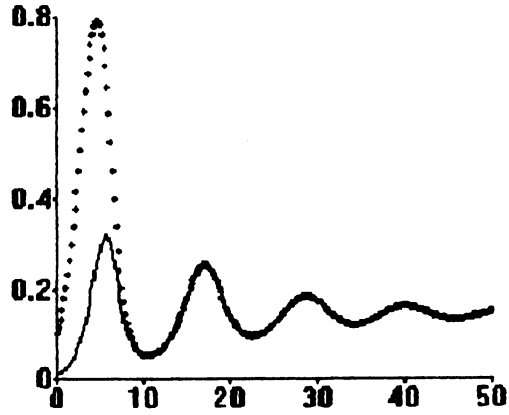

Figure 4. Graphic of tumor volumes. Dot line – primary tumor, continuous one – the secondary.

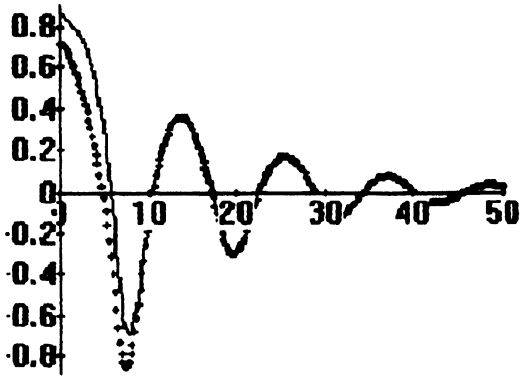

Figure 5. Graphic of specific growth rates of tumor volumes.

numbers $(x_1(t), x_2(t))$ and lymfocyte numbers in corresponding tumor $(y_1(t), y_2(t))$ with cytotoxic cell migration between two tumors (dimensionles form):

$$x_i = j_i + \frac{\alpha_1 x_i y_i}{1 + y_i} - \beta_i x_i y_i - \gamma_i x_i - c_i x_i + c_k x_k;$$

$$y_i = y_i\left(1 - x_i - \frac{\mu_i}{1 + \nu_i y_i}\right), \qquad i, k = 1, 2, \quad i \neq k,$$

where α_i, β_i, γ_i, μ_i, θ_i, c_i, j_i – positive constants – parameters of i-th tumor $(i = 1, 2)$, which we supposed to be equal in both tumors. Many known results of two tumor growth may be interpretated by these equations: completly inhibited growth of the secondary tumor when the time interval between implamentations is sufficiently long, growth of metastasis with small primary tumor, dormant tumor state, etc. We tried to explain the synhronization of tumor specific growth rates with this model. The behaviour of solution of the system may be very complicated, but we present only one example of curves of two tumor volumes (Fig. 4) and of specific growth rates (Fig. 5) against time.

It is evident that these curves do not fit our experimentals results very good. We need more experimental data and, maybe, new ideas for more appropriate model. We plan new experiments *in vitro* and use of flow cytometry technique to get additional results about interaction of populations of mallignant cells and immune system, including changes in growth fractions and cell cycle parameters.

REFERENCES

[1] E. Gorelik, Concomitant tumor immunity and the resistance to a secondary tumor challenge, *Adv. Cancer Res.*, **39** (1983), 71.

[2] R. A. de Weger, B. Wilbrink, R. M. P. Moberts, D. Mans, R. Oskam, and W. den Otter, Immune reactivity in SL2 lymphoma-bearing mice compared with SL2-immunized mice, *Cancer Immunol. Immunother*, **24** (1987), 25.

[3] R. J. North, R. H. Neubauer, J. J. H. Huang, R. C. Newton, and S. E. Loveless, Interleukin 1-induced, T cell-mediated regression of immunogenic murine tumors. Requirement for an adequate level of already acquired host concomitant immunity, *J. Exp. Med.*, **168** (1988), 2031.

[4] D. Characiejus, H. F. J. Dullens and W. den Otter, Mechanisms of tumour rejection in the murine DBA/2-SL2 concomitant immunity system, *Cancer Immunol. Immunother*, **32** (1990), 179.

[5] R. A. Maas, M. J. Becker, I. S. Weimar, J. C. de Nooy, H. F. J. Dullens, and W. den Otter, Transfer of tumor immunity by both $CD4^+$ and $CD8^+$ tumor infiltrating T lymphocytes activated in vivo by IL-2 therapy of tumor bearing mice, *Immunobiology*, **188** (1993), 281.

[6] R. P. Meiss, R. D. Bonfil, R. A. Ruggiero, and C. D. Pasqualini, Histologic aspects of concomitant resistance induced by nonimmunogenic murine tumors, *J. Natl. Cancer Inst.*, **76** (1986), 1163.

[7] R. A. Ruggiero, O. D. Bustuoabad, R. D. Bonfil, R. P. Meiss, and C. D. Pasqualini, "Concomitant immunity" in murine tumours of non-detectable immunogenicity, *Br. J. Cancer*, **51** (1985), 37.

[8] R. T. Prehn, The inhibition of tumor growth by tumor mass, *Cancer Res.*, **51** (1991), 2.

[9] J. D. Murray, *Mathematical Biology*, Springer, 1990.

[10] E. I. Alekseeva and V. A. Kuznetsov, Effect of migration cells on the stability of multicentrical immune-dependent tumors, *Biofizika*, **36** 509 (in Russian).

Pirminio ir antrinio navikų „lydimojo imuniteto" modelyje augimo statistikinė analizė ir matematinis modeliavimas

R. Eidukevičius, D. Characiejus

Tiriamas pirminio ir antrinio (implantuoto dviem dienomis vėliau) navikų augimas pelėse. Analogiškas eksperimentas atliktas ir su apšvitintomis rentgeno spinduliais pelėmis. Gauti duomenys apie kai kurių navikų dinamikos charakteristikų sinchronizaciją bei imuninės sistemos dalyvavimą navikų saveikoje. Remiantis šiais rezultatais buvo išanalizuoti žinomi matematiniai modeliai ir suplanuoti nauji eksperimentai patikslinto matematinio modelio sudarymui.