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Generation of Stable Antigen Loss Variants from Cloned Tumor Lines – An Example of Immunoadaptation During Metastasis

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The experiments to be presented will demonstrate a new type of tumor variant which can arise with high frequency within carefully cloned tumor cell lines. As a possible mechanisms of escape from a T cell mediated antitumor immune response, the tumor cells studied reduce the expression of the tumor antigen and pass this new antigen negative phenotype on to many subsequent cell generations. These findings are interpreted as an example of adaptation of tumor cells to their microenvironment. The relevance of this finding for tumor metastasis will be discussed.

As tumor model system we chose the chemically induced DBA/2 mouse (H-2^d) lymphoma L5178Y with the two sublines Eb and ESb (Schirrmacher et al. 1979a). We previously described morphological, functional, and antigenic differences between the parental tumor line Eb and its spontaneous variant ESb which arose in 1968 and had highly increased metastatic capacity (Schirrmacher et al. 1979b, 1980). In spite of these differences the tumor lines could be shown to be closely related (Schirrmacher and Bosslet 1980). Tumor protection experiments revealed the presence of tumor-associated transplantation antigens (TATAs) on both Eb and ESb tumor cells. These TATAs were shown to be distinct and non-cross-reactive and could be detected in vitro with the help of tumor-specific syngeneic cytotoxic T lymphocytes (CTL) (Bosslet et al. 1979).

The expression of these TATAs – as tested by CTL – was investigated on tumor cells which had metastasized from a local site (s.c.) to various internal organs, such as liver, lung, or spleen. In organ selection experiments this process of tumor cell spread was repeated several times (i.e., s.c. → organ → s.c. → organ etc.). Some of the typing results obtained with anti-Eb, anti-ESb, and anti-H-2 CTL are summarized in Table 1, others are published elsewhere (Bosslet and Schirrmacher, to be published).

While the control tumor lines were always specifically lysed, the tumor cells which had metastasized to the spleens of normal syngeneic mice could not be lysed by the antitumor CTL (see the ESb lines no. 2 and 10 and the Eb line no. 7). In contrast, tumor cells isolated under identical conditions from other internal organs (liver or lung) remained tumor antigen positive and could be specifically lysed by the respective CTL (see the ESb lines no. 3 and 11 and the Eb line no. 8). The tumor lines no 1–8 were derived from experiments performed with uncloned populations, thus allowing the interpretation of host selection of possible pre-existing antigen negative variants. The tumor lines no. 9–12 were derived, however, from a twice-cloned, antigen-positive ESb line. During spread from a s.c. site to the spleen (line no. 10) which took 10 days these tumor antigen positive cells converted to cells which could not be lysed by anti-ESb CTL. This finding was reproduced several times, not only with this line but also with another twice-cloned ESb line.

The evidence that the inability to be lysed by antitumor CTL was due to the loss of the respective tumor antigen is a follows: (1) As shown in the table, the cells could be lysed by anti H-2 CTL and were thus not generally resistant to lysis, (2) cold target competition experiments revealed that anti-ESb CTL could not bind to the spleen-derived ESb tumor lines, (3) the antigen negative variants could not induce CTL and thus did not express a new changed TATA, and (4) treatment of the cells

Table 1. Expression of tumor antigens and H-2[d] antigens by various metastasizing tumor lines

No.	Name	Cloning[a]	Selection[b]	% Specific cytotoxicity with CTL[c]		
				anti-Eb	anti-ESb	anti H-2[d]
1	ESb control	–	–	3	53	79
2	ESb-Met-SPL	–	s.c.-SPL, norm, 2×	3	8	85
3	ESb-Met-Liv	–	s.c.-Liv, norm, 2×	3	54	n.d.
4	ESb-Met-SPL	–	s.c.-SPL, nu/nu 2×	0	35	n.d.
5	ESb-Met-Liv	–	s.c.-Liv, nu/nu 2×	0	35	n.d.
6	Eb control	–	–	57	4	76
7	Eb-Met-SPL	–	s.c.-SPL, norm, 5×	0	0	78
8	Eb-Met-Liv	–	s.c.-Liv, norm, 3×	38	0	n.d.
9	ESb-Cl 32.2	+	–	6	78	85
10	ESb-Cl 32.2	+	s.c.-SPL, norm, 1×	0	7	56
11	ESb-Cl 32.2	+	s.c.-Lg, norm, 1×	2	42	60

[a] Cloning done by growing single cells in suspension culture in microtiterplates; ESb-Cl 32.2 is clone 32 recloned once. – = noncloned tumor cell population

[b] Selection performed in vivo by inoculation of tumor cells subcutaneously (s.c.); tumor cell containing organs (SPL = spleen, Liv = liver, Lg = lung) were removed from tumor bearing animals (10 days after inoculation of ESb or 25 days after inoculation of Eb or ESb-CL 32.2), cell suspensions prepared and, where indicated, inoculated again into normal (norm) or BALB/c nude (nu/nu) mice; this procedure was repeated several times as indicated; all cell lines were tested in the second tissue culture passage

[c] Percentage specific ^{51}Cr-release after 4 h coincubation of the indicated ^{51}Cr-labeled tumor lines with cytotoxic T lymphocytes (CTL) at an effector to target cell ratio of 40:1; n.d. = not done

with trypsin or neuraminidase did not uncover the tumor antigen.

The reduced expression or loss of tumor antigen on the clonal tumor cell variants seemed to depend on the presence of T lymphocytes in the host. Tumor lines derived from spleens of T cell deficient nude mice (Table 1, line no. 4) remained antigen positive. Also, the admixture of tumor-specific CTL with the tumor cells in a Winn-type assay led to a loss of tumor antigen on the tumor cells isolated eventually (after 4 weeks) from the s.c. site and from internal organs.

The antigen negativity of the clonal variants was a very stable type of changed phenotype: Spleen-derived antigen negative tumor variants passaged for prolonged periods in tissue culture (for more than 50 subsequent cell generations) remained antigen negative. The antigenic change thus differs from "antigenic modulation" which is usually of short duration.

It is very unlikely that the antigen negative variant derived from the twice-cloned ESb line was pre-existent, because we could not isolate such an antigen negative variant even from the original uncloned population. We also do not think that the variant arose by mutation because the changes could be reproduced with too high a frequency and in a time period allowing for not more than 10–20 cell generations. The antigen negative variants could have arisen during a process of immunoadaptation, where T cells reacting against the TATA might have signaled to the tumor cell to repress the biosynthesis of the corresponding tumor antigen. From the stability of the antigen negativity we conclude that the variants represent gene regulatory variants.

This new type of immunoadaption observed with cloned lines of highly metastatic tumor cells could explain why these tumor cells can grow in lymphoid organs such as the spleen and also why they can survive and eventually grow even in immunized hosts. Adaptive behavior of tumor cells may not only explain a new type of immune escape mechanism. As discussed elsewhere (Schirrmacher 1980), it could have a more general biologic significance for tumor cell behavior, in particular during the complex process of metastasis.

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