NATURAL ANTIBODY SURVEILLANCE OF NEOPLASTIC 
AND ACTIVATED CELLS

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ABSTRACT

Syngeneic murine tumor models have provided considerable evidence supporting the participation of polyclonal natural antibodies (NAb) in the resistance against tumors. Examination of cells selected for high binding of NAb or anti-IL-2 receptor antibodies, or through in vivo growth, now provides evidence for NAb recognition of activation-associated markers.

Keywords: activation markers, anti-IL-2 receptor antibodies, anti-tumor antibodies, tumor models

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RESUMEN

Los modelos singénicos de tumores murinos han proporcionado considerables evidencias que respaldan la participación de anticuerpos policlonales naturales (AcN) en la resistencia contra los tumores. El examen de células seleccionadas por la unión de alta afinidad de AcN o de anticuerpos contra el receptor de IL-2, o por medio de su crecimiento in vivo, proporciona ahora evidencias del reconocimiento por los AcN, de marcadores asociados a la activación.

Palabras claves: anticuerpos antirreceptor de IL-2, anticuerpos antitumor, marcadores de activación, modelos de tumores

Introduction

In vivo models of tumor development

Employing, in particular, the NK-resistant DBA/2 T-lymphoma L5178Y-F9 in a model mimicking early tumor development, we characterized the thymus-independent surveillance responsible for the rejection of a threshold subcutaneous (SC) tumor inoculum in syngeneic mice. Selective outgrowth of surviving tumors yielded a unified natural defense-resistant phenotype with an increased SC tumorigenicity, intravenous (IV) metastatic potential [1, 2] and intraperitoneal (IP) retention [3]. Furthermore, the sensitivity to natural antibody (NAb) and complement was reduced and the cells bound less NAb detected by flow cytometry, supporting a role for polyclonal NAb in the defense against small tumor foci in vivo [1].

Correlation studies of the in vitro NAb reactivity of different tumors showed an inverse relationship with the survival of threshold tumor inocula in vivo. In a genetic approach, mice bearing the CBA/N xid mutation for deficiency in B cells, IgM and IgG3 were more susceptible to a SC challenge of a syngeneic lymphoma [4]. Direct assessment showed that precultivation of tumor cells in serum NAb versus tumor-absorbed NAb reduced their tumorigenicity. Furthermore, IV pretreatment of mice with syngeneic serum NAb, (NH4)2SO4-precipitated fractions of NAb, purified IgG or IgM NAb, or human IVIG, reduced the incidence and/or increased the latency of syngeneic tumors injected subcutaneously [5, 6] and reduced the metastasis of tumor cells injected intravenously [7, Wang and Chow, unpublished results].

In vitro models of tumor development

In a model aimed at reversing progression and generating tumors with increased susceptibility uniquely to NAb, treatment of the L5178Y-F9 cell line with tumor-promoting 12-m-tetradecanoylphorbol-13-acetate (TPA) generated variants which were then selected for high NAb binding through fluorescence-activated cell sorting (FACS). This yielded the L5178Y-F9 TPA/NAb³ (LYNAb³) line which exhibited a decreased tumorigenicity [3]. Mimicking early changes associated with tumor induction, ras transformation of C3H 10T½ fibroblasts increased their NAb binding [5] with an inverse correlation between NAb binding and tumorigenicity. This provided evidence for a NAb-susceptible phase of oncogene-induced tumor development. In a model of preneoplasia, overexpression of protein kinase C-ß1 (PKC) in 10T½ cells increased their sensitivity to ras transformation. Their correlating increases in NAb binding and SC elimination from mice (Wang and Chow, unpublished results) extended the support of a role for NAb in immune surveillance to resistance against preneoplasia. Constitutive increases in the basal activity of PKC seen in both the ras transformation and PKC preneoplasia models argues for PKC upregulation of NAb binding structures and for NAb recognition of activated cells and cells in the early stages of neoplasia.

Materials and Methods

Cells and antibodies

Variants of the L5178Y-F9 line obtained through FACS or in vivo growth [3], (Table 1) were maintained in Fischer's medium with 10% fetal bovine serum [3]. NAb was bleed per cell from syngeneic DBA/2 mice [3]. Monoclonal antibodies (mAb) were obtained from ATCC.

Fluorescence-detected antibody binding

Pellets of 3 x 10⁶ cells were incubated in 100 µL of diluted serum NAb or mAb for 1 h at 4 °C, washed

Table 1. LS178Y-F9 variant binding of NAb and cell surface specific monoclonal antibodies.

<table>
<thead>
<tr>
<th>LS178Y-F9 variant</th>
<th>Murine NAb</th>
<th>Anti-TR murine IgG&lt;sub&gt;μ&lt;/sub&gt;</th>
<th>Anti-IL-2R rat IgM</th>
<th>Anti-CD45RA rat IgM</th>
<th>Anti-Thy1.2 rat IgG&lt;sub&gt;μ&lt;/sub&gt;</th>
<th>Anti-Ly-2.2 murine IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYSNAB&lt;sup&gt;a&lt;/sup&gt;</td>
<td>144.2 ± 18.7</td>
<td>53.3 ± 6.8</td>
<td>64.9 ± 13.6</td>
<td>63.9 ± 14.0</td>
<td>90.7 ± 16.6</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>LY178Y-F9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.7 ± 3.3</td>
<td>67.8 ± 3.4</td>
<td>21.9 ± 5.2</td>
<td>15.6 ± 3.3</td>
<td>91.6 ± 12.4</td>
<td>1.3 ± 0.7</td>
</tr>
<tr>
<td>Anti-IL-2R&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.5 ± 6.2</td>
<td>ND</td>
<td>47.9 ± 5.1</td>
<td>17.8 ± 3.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LS178Y-F9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.3 ± 6.9</td>
<td>ND</td>
<td>22.8 ± 2.4</td>
<td>21.1 ± 3.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Anti-IGM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.8 ± 12.2</td>
<td>51.0 ± 9.7</td>
<td>56.5 ± 7.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LS178Y-F9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.9 ± 13.5</td>
<td>62.9 ± 0.6</td>
<td>33.0 ± 2.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>In vivo&lt;sup&gt;c&lt;/sup&gt;</td>
<td>64.7 ± 6.9</td>
<td>ND</td>
<td>32.9 ± 9.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LS178Y-F9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.8 ± 6.6</td>
<td>ND</td>
<td>52.7 ± 10.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup>FACS selected twice for high anti-IL-2R binding.
<sup>b</sup>FACS selected once for high anti-IGM NAb binding.
<sup>c</sup>Selected 4 times through growth from a threshold SC tumor inoculum in DBA/2 mice.
ND: Not determined.

and incubated for 20 min at 4 °C in the appropriate FITC-coupled second antibody and fixed with 1% paraformaldehyde for assessment of the mean channel fluorescence by flow cytometry [3].

Results and Discussion

NAb binding to activated cells

Our panel of LS178Y-F9 variants demonstrated a correlation between NAb binding and the expression of the IL-2 receptor (IL-2R, p55 or 7D4) and CD45RA (RA3-2C2) (Table 1). Expression of exon-specific determinants of the signaling molecule CD45 distinguishes discrete functional populations of T cells [8]. High CD45RA and CD45RC expression and low tumorigenicity accompanied FACS selection of the LYNAb<sup>+</sup> line for high NAb binding, and in vivo outgrowth of LYNAb<sup>+</sup> line reversed each parameter [9]. Neuraminidase treatment increased binding by NAb, anti-CD45RA and anti-CD45RC. Thus, NAb probably binds preferentially asialo isoforms of CD45 expressing exons A and C. Corresponding changes in CD45 isoform expression were detected by anti-CD45 immunoprecipitation of lysates, with more variable exon expressed higher molecular weight products of mRNA splicing on the LYNAb<sup>+</sup> line. Therefore, high molecular weight asialo isoforms of CD45 were selected via NAb binding and were reduced during tumor development suggesting that they participate in NAb-mediated anti-tumor mechanisms.

NAb binding directly to CD45 was further suggested by the generation by others of a natural hybridoma autoantibody to CD45 [10]. Our analysis of NAb binding by the A strain YAC T lymphoma, wild type (WT), CD45 and a CD45RA-BC-transfected, revealed a lower NAb binding by the CD45 variant compared with the high and low isoform-bearing WT cell, and the CD45 transfected (Zhang, Ostergaard and Chow, manuscript in preparation). More specifically, NAb acid-eluted from LYNAb<sup>+</sup> cells incubated in normal syngeneic serum, demonstrated significant direct binding to purified CD45RABC and to a less extent to CD45RO/RB vs. 1% FBS-coated microtitre plate wells in an ELISA assay (Zhang, Ostergaard and Chow, manuscript in preparation). Even more importantly, cell preincubation with IgG NAb partially reduced the binding of anti-CD45RA > anti-CD45RB and anti-pan CD45 (Zhang, Ostergaard and Chow, manuscript in preparation). Together these approaches support CD45 as one of the cell surface molecules to which NAb binds, implicating NAb in the direct regulation of CD45 function on lymphoid tumors and normal cells (Zhang, Ostergaard and Chow, manuscript in preparation). Identification of a transitional, higher density CD45RA<sup>+</sup> naïve adult T cell phenotype 1 to 2 days after mitogenic stimulation of T cells [11] which coincides with CD45RA expression on the most highly proliferating cells [12], suggests that highly proliferating cells which appear briefly, early after activation might be exquisitely sensitive to regulation by NAb. This is supported by the correspondence of an increased expression of the IL-2R chain and CD44 on the LYNAb<sup>+</sup> cell line (Wang and Chow, unpublished data), which is consistent with increases in IL-2R and CD44 on activated T cells. Furthermore, other investigators have shown that a tumor-binding IgM mNAb bound to activated T cells [13] and natural human serum IgG reacted with PHA-activated T cells completely inhibiting their stimulation of autologous T cells in an autologous mixed lymphocyte reaction [14]. These observations argue for the hypothesis that NAb regulates cells undergoing activation, providing a unifying concept for NAb activity against cells including their role in the homeostasis of the organism, regulating the immune response and defending against tumor development and invading pathogens. This opens a new phase of research toward an understanding of NAb functions from all perspectives including biochemical, physiological, developmental, pathological, diagnostic and therapeutic.


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