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

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 preincubation of tumor cells in serum NAb vs tumor-absorbed NAb reduced their tumorigenicity. Furthermore, IV pretreatment of mice with syngeneic serum NAb, $(NH_4)_2SO_4$-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/NAb⁺3 (LYNAb⁺) 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 preneoplasm, overexpression of protein kinase C-β1 (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 preneoplasm. Constitutive increases in the basal activity of PKC seen in both the ras transformation and PKC preneoplasm models argues for PKC upregulation of NAb binding structures and for NAb recognition of activated cells and cells in the early stages of neoplasm.

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 bled per axilla from syngeneic DBA/2 mice [3]. Monoclonal antibodies (mAb) were obtained from ATCC.

Fluorescence-detected antibody binding

Pellets of 3×10^5 cells were incubated in 100 μL of diluted serum NAb or mAb for 1 h at 4 °C, washed

1. Brown GW, Lesiuk T, Chow DA. Phenotypic alterations in tumors that develop from threshold subcutaneous inocula. I. Reduced binding of natural antibodies and sensitivity to hypotonic lysis. J Immunol 1986;136:3116-23.

2. Reese MR, Chow DA. Tumor progression *in vivo*: Increased SBA lectin binding, N-acetylgalactosamine-specific lectin expression and liver metastasis potential. Cancer Res 1992;52:5235-43.

3. Tough DF, Chow DA. Tumorigenicity of murine lymphomas selected through fluorescence-detected natural antibody binding. Cancer Res 1988;48:270-5.

4. Chow DA, Bennet RD. Low natural antibody and low *in vivo* tumor resistance in xid-bearing deficient mice. J Immunol 1989;142:3702-6.

5. Tough DF, Haliotis T, Chow DA. Regulation of natural antibody binding and susceptibility to natural killer cells through Zn^{++}-inducible ras oncogene expression. Int J Cancer 1992;50:423-30.

6. Chow DA. Reduced tumorigenicity of threshold tumor inocula in xid-bearing mice treated with natural antibodies. Int J Cancer 1995;60:848-53.

7. Chow DA, Yuan X-Y, Tough DF. Polyclonal natural antitumor antibody binding dynamics: Preferential release of surface membrane molecules and increased metastasis. Inv & Met 1992; 12:218-32.

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Table 1. L5178Y-F9 variant binding of NAb and cell surface specific monoclonal antibodies.

L5178Y-F9 variant	Mean channel fluorescence ± SE					
	Murine NAb	Anti-TrR murine IgG$_{2b}$	Anti-IL-2R rat IgM	Anti-CD45RA rat IgM	Anti-Thy1.2 rat IgG$_{2a}$	Anti-Lyt2.2 murine IgM
LYNAb⁺	144.2 ± 18.7	53.3 ± 6.8	64.9 ± 13.6	63.9 ± 14.0	90.7 ± 16.6	1.8 ± 0.8
L5178Y-F9	63.7 ± 3.3	67.8 ± 3.4	21.9 ± 5.2	15.6 ± 3.6	91.6 ± 12.4	1.3 ± 0.7
Anti-IL-2R⁺ᵃ	76.5 ± 6.2	ND	47.9 ± 5.1	17.8 ± 3.7	23.2 ± 10.5	0.8 ± 0.5
L5178Y-F9	58.3 ± 6.9	ND	22.8 ± 2.4	21.1 ± 3.1	36.5 ± 13.5	1.3 ± 0.7
Anti-IgM⁺ᵇ	96.8 ± 12.2	51.0 ± 9.7	56.5 ± 7.6	ND	ND	ND
L5178Y-F9	74.9 ± 13.5	62.9 ± 0.6	33.0 ± 2.9	ND	ND	ND
In vivo 4XSCᶜ	64.1 ± 6.9	ND	32.9 ± 9.6	ND	ND	ND
L5178Y-F9	75.8 ± 6.6	ND	52.7 ± 10.6	ND	ND	ND

ᵃFACS selected twice for high anti-IL-2R binding.
ᵇFACS selected once for high anti-IgM NAb binding.
ᶜSelected 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 L5178Y-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 signalling molecule CD45 distinguishes discrete functional populations of T cells [8]. High CD45RA and CD45RC expression and low tumorigenicity accompanied FACS selection of the LYNAb⁺ line for high NAb binding, and in vivo outgrowth of LYNAb⁺ 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 encoded higher molecular weight products of mRNA splicing on the LYNAb⁺ 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 CD45RABC-transfectant, revealed a lower NAb binding by the CD45⁻ variant compared with the high and low isoform-bearing WT cell, and the CD45 transfectant (Zhang, Ostergaard and Chow, manuscript in preparation). More specifically, NAb acid-eluted from LYNAb⁺ 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 microtiter 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⁺ naive 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⁺ 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.

8. Trowbridge IS, Thomas ML. CD45: an emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. Annu Rev Immunol 1994;12:85-116.

9. Zhang Z, Chow DA. Differential CD45 isoform expression accompanies reduced natural antibody binding in L5178Y-F9 tumor progression. J Immunol 1997;159:344-50.

10. Glotz D, Zanetti M. A natural neonatal hybridoma antigen autoantibody to the T200 antigen. J Immunol 1989;142:439-43.

11. Deans JP, Serra HM, Shaw J, Shen YJ, Torres RM, Pilarski LM. Transient accumulation and subsequent rapid loss of messenger RNA encoding high molecular mass CD45 isoforms after T cell activation. J Immunol 1992;148:1398-905.

12. Johannisson A, Festin R. Phenotype transition of CD4⁺ T cells from CD45RA to CD45RO is accompanied by cell activation and proliferation. Cytometry 1995;19:343-52.

13. Bohn J, Roggenbuck D, Settmacher U, Docke W, Volk H-D, Von Baehr, et al. Binding of natural human auto-antibodies to human tumor cell lines and stimulated normal T lymphocytes. Lett 1994;39:187-94.

14. Wolf-Levin R, Azuma T, Aoki K, Yagami Y, Okada H. Specific IgG autoantibodies are potent inhibitors of autoreactive T cell response to phytohemagglutinin-activated T cells. J Immunol 1993;151:5864-77.