Table 2. Effects of *SIP1* gene dosage and mutations on invertase expression. Invertase activity is expressed as micromoles of glucose released per minute per 100 mg (dry weight) of cells. Values are averages from two to four strains (four for *snf4* strains). For values >1, standard errors were <25%.

Relevant genotype	Plasmid-borne gene	Temperature (°C)	Invertase activity	
			R	D
snf4Δ (YEp24)	None	30°	<1	4*
snf4Δ (pB44)	*SIP1*	30°	<1	37*
snf4Δ (pLN132)	*SNF4*	30°	<1	140*
snf1Δ (pB44)	*SIP1*	30°	<1	<1*
sip1Δ		23°	1	190†
snf4Δ		23°	<1	27†
sip1Δsnf4Δ		23°	<1	12†

*Cultures were grown at 30°C in synthetic complete Ura with 2% glucose (repressing conditions, R) and shifted to 0.05% glucose for derepression (D) (*6*). †Cultures were grown and derepressed in rich medium at 23°C, a semipermissive temperature for *snf4Δ* mutants (*7*).

coprecipitating proteins, and assays of a mutant altered in the conserved Lys of the SNF1 adenosine triphosphate (ATP) binding site (*snf1-K84R*) showed that the phosphorylation is dependent on SNF1 activity (6) (Fig. 4A). One of the phosphorylated products detected in an assay of a wild-type extract migrates as a 110-kD protein, which is approximately the size predicted for SIP1. This phosphoprotein was not detected in assays of an *sip1Δ* mutant (Fig. 4, A and B), suggesting that this protein corresponds to SIP1. We constructed a plasmid expressing a LexA-SIP1 fusion protein (Fig. 2), which was detected as a 130-kD protein on protein immunoblots. In an assay of an *sip1Δ* mutant carrying this plasmid, a new phosphoprotein corresponding to LexA-SIP1 was detected (Fig. 4A). These results show that the SIP1 protein co-immunoprecipitates with SNF1 and is phosphorylated in vitro. The diffuse nature of the band suggests that SIP1 is heterogeneously modified. The absence of phosphorylation in immune complexes containing the SNF1-K84R mutant protein indicates that phosphorylation of SIP1 requires SNF1 kinase activity.

Increased expression of SIP1 does not appear to suppress *snf4* defects by stimulating SNF1 kinase activity. Multiple copies of *SIP1* do not restore SNF1 kinase activity in an *snf4Δ* mutant but do allow weak detection of phosphorylated SIP1 protein (Fig. 4B). Taken together, our results suggest that SIP1 functions downstream of SNF1. One possibility is that SIP1 mediates the effects of the SNF1 kinase in the signaling pathway. Alternatively, SIP1 could facilitate interaction of SNF1 with other substrates.

These genetic and biochemical studies show that the interaction between SIP1 and the SNF1 protein kinase detected by the two-hybrid method reflects a functional interaction in vivo. The method is applicable to cloned kinase genes from any organism and is very sensitive; interactions yielding only a few units of β-gal activity can be detected in the color assay. Thus, the two-hybrid system should prove generally useful for identifying substrates and other proteins that interact with protein kinases.

REFERENCES AND NOTES

1. S. Fields and O.-K. Song, *Nature* **340**, 245 (1989).
2. C.-t. Chien, P. L. Bartel, R. Sternglanz, S. Fields, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9578 (1991).
3. J. L. Celenza and M. Carlson, *Science* **233**, 1175 (1986).
4. S. Thompson-Jaeger, J. Francois, J. P Gaughran, K. Tatchell, *Genetics* **129**, 697 (1991).
5. A. Alderson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8602 (1991).
6. J. L. Celenza and M. Carlson, *Mol. Cell. Biol.* **9**, 5034 (1989).
7. J. L. Celenza, F. J. Eng, M. Carlson, *ibid.*, p. 5045.
8. Three pGAD libraries (*2*) of plasmids carrying fusions between GAL4(768-881) and yeast *Sau3*AI fragments, in each reading frame, were used to transform (*11*) yeast strain GGY::171 [G. Gill and M. Ptashne, *Cell* **51**, 121 (1987)], which is deleted for *GAL4* and contains *GAL1-lacZ*, carrying the GAL4(1-147)-SNF1 hybrid plasmid (*1*). Transformants on plates selective for the plasmids and containing glucose were replicated onto nitrocellulose filters and were incubated with the chromogenic substrate X-Gal [J. Breeden and K. Nasmyth, *Cold Spring Harbor Symp. Quant. Biol.* **50**, 643 (1985)]. Out of 144 blue colonies, 82 turned blue on reassaying after purification on selective medium. Of these, 27 colonies containing *GAL4* plasmids were identified by the polymerase chain reaction with primers complementary to nucleotides 1206 to 1229 and 2552 to 2528 of the *GAL4* sequence and template prepared by boiling yeast colonies. For the remaining 55 colonies, cultures were grown in medium lacking Leu, DNA was prepared, and the *LEU2*-marked library plasmid was recovered by transformation of the *leuB*⁻ *Escherichia coli* strain BA1 to ampicillin resistance and Leu independence. Each plasmid was used to transform GGY::171 with and without GAL4(1-147)-SNF1. Sixteen plasmids caused blue colony color only in the presence of GAL4(1-147)-SNF1, ten plasmids conferred blue color independent of GAL4(1-147)-SNF1, and in the remaining cases the recovered plasmid did not cause a blue phenotype. We compared the 16 plasmids by colony hybridization of the bacterial clones (*14*). The plasmids pG_{AD}-$SIP1_{201}$ and pG_{AD}-$SIP1_{432}$ (named according to the codon at the fusion point) were from different libraries.
9. M. Carlson and D. Botstein, *Cell* **28**, 145 (1982).
10. E. J. A. Hubbard, X. Yang, M. Carlson, *Genetics* **130**, 71 (1992); L. C. Robinson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 28 (1992).
11. Standard yeast genetic methods were used throughout [M. D. Rose, F. Winston, P. Hieter, *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1990)].
12. J. Ma and M. Ptashne, *Cell* **51**, 113 (1987).
13. L. Guarente, *Methods Enzymol.* **101**, 181 (1983); J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1972).
14. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989).
15. D. M. Ruden, J. Ma, Y. Li, K. Wood, M. Ptashne, *Nature* **350**, 250 (1991).
16. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).

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Participation in Normal Immune Responses of a Metastasis-Inducing Splice Variant of CD44

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A variant of the glycoprotein CD44 (CD44v) that shares sequences with variants causally involved in metastasis formation is transiently expressed on B and T lymphocytes and macrophages after antigenic stimulation and in the postnatal period. Antibodies to the variant hinder in vivo activation of both B and T cells. The observation that a protein domain that is expressed on CD44 and required for the lymphatic spread of tumor cells can catalyze an essential step in the process of lymphocyte activation supports the idea that metastasizing tumor cells mimic lymphocyte behavior.

Tumor cells that metastasize by way of the lymphatics leave the site of local tumor growth and enter peripheral lymphatic organs through the afferent lymph. This process resembles the trafficking of lymphocytes. When lymphocytes contact antigen, they leave the periphery and enter the draining lymphatic tissue where they are selectively retained, activated, and expanded before reentry into the circulation (*1*).

We have recently observed that highly

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metastatic rat tumor cells (BSp73ASML cells) (*2*) express splice variants of the glycoprotein CD44 (CD44v) (*3*). Antibodies to the common variant portion of these variants block the settlement of the tumor cells in the draining lymph nodes and block consequently any further spread (*3, 4*). When individually expressed, two of these variants confer full metastatic behavior to nonmetastasizing tumor cells (*3, 5*).

The similarity of these processes—lymphogenic tumor spread and lymphocyte retention and activation after antigen contact—prompted us to explore whether a splice variant of CD44 had a physiologic function during lymphocyte activation.

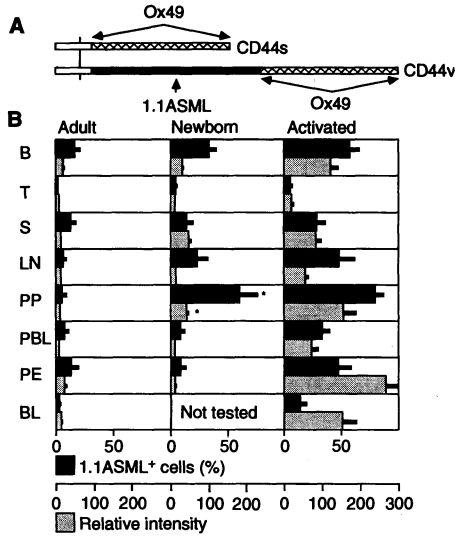

Fig. 1. Expression of CD44v on lymphoid cells. Cells from various lymphoid organs were analyzed by immunofluorescence for the expression of CD44s (MAb Ox49) (*8*) and of a variant of CD44 that carries the epitope for the MAb 1.1ASML (*3, 9*). (**A**) The approximate positions of the epitopes on CD44s and on the variant isolated from the metastatic tumor. The variant shown carries exon sequences of v4 through v7 (Fig. 3). Surface expression of the epitopes was determined with MAbs Ox49 [immunoglobulin G2a (IgG2a)] and 1.1ASML (IgG1) as the first antibodies and phycoerythrin-labeled goat antibody to mouse IgG2a and fluorescein isothiocyanate–labeled goat antimouse IgG1 as second antibodies. In a presetting with BSp73-ASML, fluorescence intensities of both dyes had been adjusted to comparable levels. Staining was determined by a fluorescence-activated cell sorter (FACS) scanner (EPICS 752 Dye Laser System, Coulter, Krefeld, Germany). All cells stained heavily with Ox49 (100 to 1000 relative intensity) (*8*). (**B**) Plot of the number of 1.1ASML-positive cells and their average intensity. The values represent the means of three independent experiments with one rat each. The standard deviations are indicated. Adult, pathogen-free BDX rats were 8 weeks old (Charles River, Sulzfeld, Germany); newborn rats were 24 hours old. "Activated" represents cells from adult rats that had received 1×10^8 irradiated (3000 R) allogeneic (DA strain) lymphocytes subcutaneously and intraperitoneally 5 days before analysis. B, bone marrow; T, thymus; S, spleen; LN, lymph node; PP, Peyer's patches; PBL, peripheral blood lymphocytes; PE, peritoneal exudate; and BL, bronchial lavage. Asterisks indicate mesenteric LN.

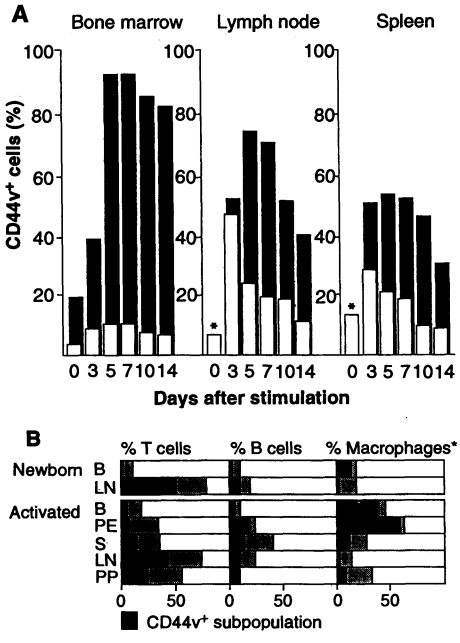

Fig. 2. (**A**) Time course of antigen-induced CD44v expression. Cells from bone marrow, lymph nodes, and spleen of one adult BDX rat each were harvested at various times after stimulation in vivo by allogeneic cells (Fig. 1). The cells were sorted by FACS scan according to size and epitope. Forward and sideward scatter revealed two population of cells that differed in size (log forward scatter) and granulation (log sideward scatter). The number of MAb 1.1ASML–positive small (white bars) and large (black bars) cells were plotted versus time. One representative experiment is shown; the experiment was repeated three times with similar results. Asterisks indicate where large cells were not detectable. (**B**) Distribution of CD44v-positive hematopoietic cells. Lymphoid organs were collected from newborn rats (within 24 hours after birth) and from 8-week-old (adult) antigenically stimulated BDX rats (Fig. 1). Lymphocytes were double-stained with MAb 1.1ASML and MAb Ox52 (T cells and thymocytes) (*14*), anti-rat IgM (μ chain–specific), or MAb Ox41 (asterisk indicates macrophages and dendritic cells and precursor cells in the bone marrow) (*15*). The percentage of cells staining only with an antibody to either the T cell, B cell, macrophage, or dendritic cell markers (light gray bars) and the percentage of cells double-staining with one of these antibodies and MAb 1.1ASML (dark gray bars) are plotted. Data from one experiment are shown; two other experiments yielded similar results. Abbreviations are as in Fig. 1.

Lymphocytes have been known to express the standard type of CD44 (CD44s) that is thought to facilitate lymphocyte emigration from the blood stream at the sites of high endothelial venules (*6, 7*). We report here that after lymphocytes contact antigen, they transiently express a variant of CD44 and that this variant is involved in B and T cell activation in vivo.

To screen lymphatic tissue for CD44v expression, we used the metastasis-(CD44v)–specific monoclonal antibody (MAb) 1.1ASML and MAb Ox49, which recognizes an epitope on both CD44s and CD44v (Fig. 1A). Although more than 80% of the lymphocytes from all tissues tested (Fig. 1B) were stained with Ox49 at a high fluorescence intensity (*8*), the adult rats we used expressed almost no 1.1ASML epitope (Fig. 1B). Immediately after the rats were born, however, a large fraction of their cells, particularly from bone marrow and mesenteric lymph nodes, was positive for 1.1ASML, which indicates expression of a variant of CD44 (Fig. 1B).

The early appearance of $CD44v^+$ cells in the postnatal period and particularly in mesenteric lymph nodes may depend on contact with external antigen or may be restricted to a subset of naturally activated lymphocytes. We tested the assumption of CD44v expression during lymphocyte activation by injecting allogeneic cells into adult rats. The percent of $CD44v^+$ cells increased in most lymphatic tissues, particularly in bone marrow, spleen, lymph nodes, Peyer's patches, and peritoneal exudate (Fig. 1B). The increase in staining intensity and the number of positive cells was transient; maximal numbers were reached 3 to 5 days after antigen injection (Fig. 2A).

During the activation process, the small peripheral lymphocytes become blast cells. If the expression of CD44v were related to the activation process, the epitope should appear either just before or with the blast cell stage. To measure the size distribution, we sorted the cells according to size and granulation. Whereas CD44s was equally expressed in all cells irrespective of size (*8*), the 1.1ASML epitope was detected only in a minority of small cells; most positive cells were large (Fig. 2A). Particularly in cells from lymph nodes and spleen, the peak of expression in small cells preceded that in large cells, which is compatible with the idea that CD44v expression is an early event in lymphocyte activation.

To determine which cells become $CD44v^+$ in both the newborn period and upon allogeneic antigen injection, we double-stained the cells with MAb 1.1ASML and with MAbs specific for subsets of leukocytes (Fig. 2B). CD44v was found on macrophages, T cells, and B cells, and the

relative proportions varied with the source of lymphoid tissue. In lymph nodes, mainly T cells were positive for CD44v, whereas in the peritoneal cavity, expression of CD44v was restricted to B cells and cells of the macrophage lineage (Fig. 2B). Thus, all classes of cells of the immune system that are in a process of activation probably express CD44 variants.

These data are compatible with previous Northern (RNA) blot experiments that demonstrated a specific hybridization with our CD44v cDNA clone of RNA in a minor fraction of cells in lung, spleen, and lymph nodes (*3*). Whereas the metastasizing rat tumor cells express a whole variety of different splice variants, these cells produced only one RNA splice product (*3*). Thus, metastatic cells may have a splice disorder, or several splice variants may be involved in the metastatic process, whereas the physiologically regulated splice probably yields one or a few defined products. Cells can choose from at least ten variant exons (*9, 10*). In order to determine which RNAs were produced in activated lymphocytes, we labeled cells with MAb 1.1ASML, sorted the positive cells from bone marrow and lymph nodes, and analyzed the RNAs by reverse transcription polymerase chain reaction (PCR) (Fig. 3). With primers from outside the variant exon sequences (Fig. 3, primers A and D), CD44s and the variants can be determined; the predominant splice product was that of CD44s. To accurately delimit the variant sequences that carried the epitope 1.1ASML, we used nested primers (Fig. 3, primer B for the 5′ side and primer C for the 3′ side). The variant expressed in cells sorted from lymph nodes contained only sequences of exon v6 (Fig. 3). This is a variant of CD44 which has not yet been cloned, but which shares the 1.1ASML epitope-containing exon v6 sequence with CD44v in metastatic tumor cells. In sorted bone marrow cells, several variants are expressed, including the lymph node variant; the other variants are larger. It is not clear whether different variants are expressed in different subpopulations of cells and what their functional significance is.

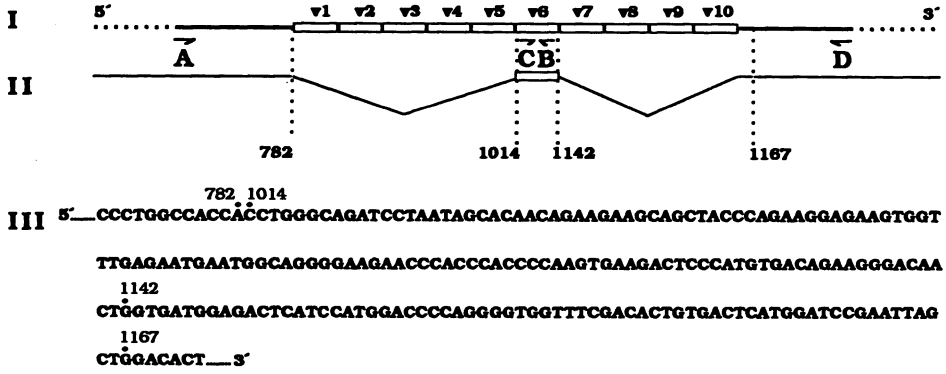

Fig. 3. Sequence representing the variant expressed in activated lymphocytes isolated from lymph nodes. The positions refer to those given for pMeta-1 [figure 4 in (*3*)]. The cells from bone marrow and lymph node of DA rats that were sorted had been obtained from adult animals 5 days after injection of allogeneic irradiated BDX lymphocytes as described (Fig. 1). Total RNA was prepared and used as template for reverse PCR. With respect to the numbering of the sequence of pMeta-1 [figure 4 in (*3*)], the oligonucleotides for priming have the following positions: oligo A, 102 to 131; oligo B, 1103 to 1132; oligo C, 1020 to 1047; and oligo D, 1549 to 1571. After 45 cycles between primers A and D, further amplification was performed (25 cycles) with nested primers of sequences within the epitope-encoding exon. Primers A and B permit exact delimitation of the splice variant on the 5′ side of the epitope-encoding exon, and primers C and D yield the corresponding information on the 3′ side. All amplification products were sequenced. We suggest that the variant exons be numbered v1 through v10 (I). The putative exon structure of the CD44 gene has been derived from numerous PCR analyses of different splice variants and from a map of murine genomic clones (*10*). The lymphocytes contain CD44s RNA and RNA of a variant with v6 only (II and III). In contrast to pMeta-1, standard type-specific sequences coding for 23 amino acids as shown [figure 9 in (*3*)] are part of the molecule.

The transient nature of CD44v expression in response to antigenic stimulation suggested a functional role of CD44v in lymphocyte activation. The function of CD44v in the metastatic process can be blocked by subcutaneous or intravenous injection of MAb 1.1ASML (*4, 11*). With this, the formation of metastases in lymph nodes was retarded or prevented. This encouraged us to attempt to interfere with CD44v function during the activation of lymphocytes by injecting MAb 1.1ASML. We injected purified MAb (200 μg) simultaneously either with trinitrophenol conjugated to lipopolysaccharide (TNP-LPS) or horse red blood cells (TNP-HRBCs) or with allogeneic lymphocytes and determined, at the days indicated (Table 1 and Fig. 4), the number of antigen-specific, plaque-forming cells and the antibody titer or the generation of cytotoxic cells.

The antibodies reduced both the T cell–dependent and T cell–independent B lymphocyte response (Table 1). In the presence of 1.1ASML, the yield of hapten and carrier-specific plaques and the hapten-specific antibody titer dropped. In three independent experiments with TNP-LPS, the reduction of hapten-specific plaques was 36, 38, and 8%. In the latter experiment, the antibody titer was reduced to 5% (Table 1). In two experiments with TNP-HRBCs, the TNP-specific plaques were reduced to 15 and 9% and carrier-specific plaques were reduced to 21%. To ascertain that the effect of MAb 1.1ASML was a result of blockage of a specific function of CD44v (exon v6) and not a result of Fc-mediated suppression, we prepared $F(ab')_2$ fragments and injected them instead of whole immunoglobulin. The $F(ab')_2$ fragments also inhibited the B cell activation (Table 1), which indicates

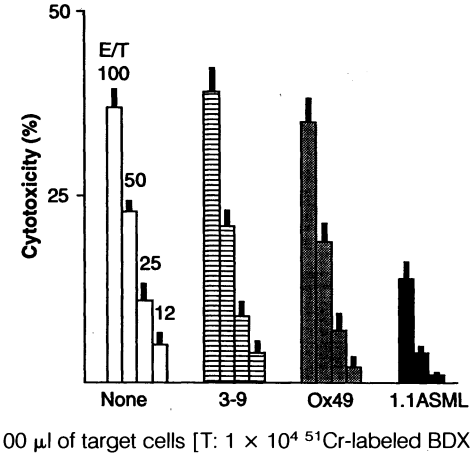

Fig. 4. Influence of antibodies directed against the variant portion (v6) of CD44v upon activation of CTLs. For each experiment, three DA rats were immunized with 5×10^7 irradiated (3000 R) BDX lymphocytes each and received concomitantly, where indicated, an intravenous injection of 100 μg of protein A–Sepharose 4B (Pharmacia, Freibürg, Germany) affinity-purified MAb 1.1ASML, MAb Ox49, or MAb 3-9 (anti-unrelated hapten). Spleen cells were collected 7 days after immunization and were tested for cytotoxic activity against BDX blasts immediately. They were adjusted to 1×10^7 cells per milliliter of RPMI-1640 medium supplemented with L-glutamine, antibiotics, 10^{-3} M Hepes buffer, and 5% heat-inactivated fetal calf serum. One hundred microliters of cell suspension (E: effector cells) was titrated in U-bottomed microtiter plates, and 100 μl of target cells [T: 1×10^4 ^{51}Cr-labeled BDX concanavalin A (Pharmacia, Freibürg, Germany) blasts] was added. After 6 hours of incubation at 37°C, plates were centrifuged, aliquots of supernatants were harvested, and the amount of ^{51}Cr released was determined in a gamma counter. The mean percentage of specific release and the standard deviations were calculated from three such experiments (each using three rats).

Table 1. Antibodies directed against CD44v block B lymphocyte activation. DA rats (five per group) were immunized intraperitoneally with 50 μg of TNP-LPS (*16*) or 5 × 10^8 TNP-HRBCs (*17*). Plaque-forming cells (PFCs) were determined 3 and 5 days later, respectively, with TNP-SRBCs (TNP coupled to sheep red blood cells) and HRBCs as target cells. PFCs were determined according to a modification of the hemolytic plaque assay (*18, 19*). MAbs (200 μg) or $F(ab')_2$ fragments (100 μg) were injected intravenously, concomitantly with the antigen, as indicated. I, II, and III refer to three independent experiments. Each figure represents the mean of data from five rats. Quantitation of anti-TNP antibodies in the serum of immunized rats was performed as described (*18*) by comparison of serum titration curves with standard curves of purified anti-TNP MAb in an enzyme-linked immunosorbent assay (*20*). NT, not tested.

Antigen	Antibody	PFC/10^6 spleen cells				Serum
		Anti-TNP			Anti-HRBC	Anti-TNP (μg/ml)
		I	II	III		
TNP-LPS	3-9 (IgG1)	776 ± 32	525 ± 39	620 ± 45		108.0 ± 25.1
	1.1ASML (IgG1)	280 ± 41	197 ± 51	52 ± 23		5.1 ± 3.1
	1.1ASML-$F(ab')_2$	459 ± 87	290 ± 49	NT		NT
	Ox49	502 ± 51	443 ± 31	NT		NT
TNP-HRBC	3-9 (IgG1)	219 ± 23		214 ± 31	1582 ± 89	
	1.1ASML (IgG1)	33 ± 8		20 ± 9	337 ± 55	
	1.1ASML-$F(ab')_2$	72 ± 11		NT	605 ± 74	

that it is the specific function of the CD44 variant that is involved in lymphocyte activation.

The alloantigen-induced proliferative T cell response (*8*) and the generation of cytotoxic T cells (CTLs) (Fig. 4) were inhibited by 1.1ASML; the control MAb antibody had no effect in CTL activation. Because we have no MAbs that recognize CD44s exclusively, it is difficult to predict how a MAb to the CD44s portion of CD44v should act (*12*). We tested MAb Ox49 for both the B cell and the CTL response and found less pronounced or weak interference (Fig. 4). This suggests that the epitope of MAb Ox49 is sufficiently far from the domain of CD44v that is important for the immune response.

Antigen-presenting cells and lymphoid cells meet antigen in the periphery. The expression of CD44v triggered in these cells is involved in the early phase of the primary immune response. Metastatic tumor cells can use variants sharing exon v6 in the spread to lymph nodes. This suggests that metastatic cells, by mimicking lymphocytes, recruit a physiologic genetic program. The inhibition by antibody of the lymphogenic spread of tumor cells and of the immune response is compatible with the idea that CD44v with the exon v6 participates in a relevant interaction within or before entry into the lymphoid organs (*13*). This interaction may be useful in the therapeutic modulation of the immune system.

REFERENCES AND NOTES

1. J. L. Gowans and E. J. Knight, *Proc. R. Soc. London, Ser. A* **159**, 282 (1964).
2. S. Matzku, D. Komitowski, M. Mildenberger, M. Zöller, *Invasion & Metastasis* **3**, 109 (1983).
3. U. Günthert *et al.*, *Cell* **65**, 13 (1991).
4. S. Seiter, R. Arch, D. Komitowski, M. Hofmann, H. Ponta, P. Herrlich, S. Matzku, M. Zoller, unpublished data.
5. W. Rudy, M. Hofmann, R. Schwartz-Albiez, M. Zoller, K.-H. Heider, H. Ponta, P. Herrlich, unpublished data.
6. I. S. Trowbridge *et al.*, *Immunogenetics* **15**, 299 (1982).
7. S. Jalkanen, R. Bargatze, L. Herron, E. C. Butcher, *Eur. J. Immunol.* **16**, 1195 (1986).
8. R. Arch, K. Wirth, M. Hofmann, H. Ponta, S. Matzku, P. Herrlich, M. Zöller, data not shown.
9. M. Hofmann *et al.*, *Cancer Res.* **51**, 5292 (1991).
10. C. Tölg, M. Hofmann, H. Ponta, P. Herrlich, unpublished data.
11. S. Reber *et al.*, *Int. J. Cancer* **46**, 919 (1990).
12. The antibody could interfere with the function of either CD44v or CD44s, both of which are expressed on activated lymphocytes. CD44s is also found in resting lymphocytes and appears to be engaged in the adhesion of lymphocytes to endothelial cells [S. Jalkanen, A. C. Steere, R. I. Fox, E. C. Butcher, *Science* **233**, 556 (1986); W. M. Gallatin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4654 (1989); R. L. Idzerda *et al.*, *ibid.*, p. 4659]. Inhibition of this interaction may also inhibit the natural immune response. On the other hand, certain CD44s-recognizing antibodies have been reported to stimulate lymphocytes in vitro to proliferation [(*14, 15*); S. Huet *et al.*, *J. Immunol.* **143**, 798 (1989); S. M. Denning, P. T. Le, K. H. Singer, B. F. Haynes, *ibid.* **144**, 7 (1990); Y. Shimizu, G. A. van Seventer, R. Siraganian, L. Wahl, S. Shaw, *J. Immunol.* **143**, 2457 (1989)].
13. The MAb 1.1ASML does not down-modulate expression of CD44v (*8*). We therefore favor the explanation that this represents interference with a functional interaction. Such functional interference may account for the long-lasting allograft tolerance that is induced when antibodies to both intercellular adhesion molecule–1 and leukocyte function–associated antigen–1 are administered [M. Isobe, H. Yagita, K. Okumura, A. Ihara, *Science* **255**, 1125 (1992)].
14. A. P. Robinson, M. Puklave, D. M. Mason, *Immunology* **55**, 527 (1985).
15. A. P. Robinson, T. M. White, D. W. Mason, *ibid.* **57**, 239 (1986).
16. I. J. Fidler, *Cell. Immunol.* **16**, 223 (1975).
17. M. R. Rittenberg and H. L. Pratt, *Proc. Soc. Exp. Biol. Med.* **132**, 575 (1969).
18. N. K. Jerne and A. A. Nordin, *Science* **140**, 405 (1963).
19. M. Zoller, *Scand. J. Immunol.* **31**, 619 (1990).
20. E. Engvall and P. Perlman, *J. Immunol.* **109**, 129 (1982).

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