Review Article

Murine leukemia RL♂1 and sarcoma Meth A antigens recognized by cytotoxic T lymphocytes (CTL)

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Peptide elution and expression cloning methods have been used to identify T cell-recognized antigens for which no molecular information is available. We identified a unique tumor antigen peptide pRL1a, IPGLPLSL that is recognized by CTL on BALB/c RL♂1 leukemia by peptide elution. The sequence of the peptide corresponded to the normally untranslated 5′ region of *akt*. Cytotoxicity was generated in BALB/c spleen cells by *in vivo* and *in vitro* sensitization with pRL1a peptide in the form of multiple antigen peptide (MAP), but not the original form. pRL1a MAP immunization had a significant growth-inhibitory effect. pRL1a MAP was mostly internalized into the endosomal compartment of antigen-presenting cells, leaked to the cytosol, and degraded, and the pRL1a peptide produced was presented through the MHC class I pathway. *In vivo* depletion of CD4 T cells from tumor-inoculated BALB/c mice caused RL♂1 regression. Overexpression of the RLakt molecule seemed to induce CD4 immunoregulatory cells, which resulted in progressive RL♂1 growth in BALB/c mice. *In vivo* administration of anti-CD25 mAb (PC61) caused regression of RL♂1, suggesting that $CD4^+CD25^+$ immunoregulatory cells were involved in the tumor growth. Recently, we improved the sensitivity and the efficacy of T cell antigen cloning from cDNA expression libraries by using large- and small-scale ELISPOT assays. Using the IFN-γ ELISPOT method, we obtained a cDNA clone S35 of 937 bp recognized by AT-1 CTL on BALB/c Meth A sarcoma. S35 was a part of the retinoic acid-regulated nuclear matrix-associated protein (ramp). AT-1 CTL recognized the peptide LGAEAIFRL, which was derived from a newly created open reading frame due to the exon 14 extension. (Cancer Sci 2003; 94: 931–936)

Tumor rejection antigens were first found on methylcholanthrene-induced fibrosarcomas in mice.[1–5] Immunization of syngeneic mice with a tumor was shown to render these mice resistant to successive challenge by the same tumor. Studies on adoptive transfer showed that the tumor rejection response is mediated by T cells.[6, 7] CD8 T cells are predominantly responsible for rejection, while CD4 T cells are involved to various extents, depending on the tumor. It is generally accepted that CD4 T cells help CD8 CTL precursors (pCTL) to differentiate into effector cells.[8, 9] CD8 and CD4 T cells recognize the peptide derived from intracellular and extracellular proteins presented on major histocompatibility complex (MHC) class I and class II molecules, respectively.

Radiation-induced leukemia and methylcholanthrene-induced sarcoma occur after long latency and have been used as model tumors of human malignancies. RL♂1 is a radiation-induced leukemia[10] and Meth A is a methylcholanthrene-induced sarcoma in BALB/c mice.[11] Both those tumors were established in 1963 by Lloyd J. Old and have been widely used for the study of tumor immunology in many laboratories. Here, we describe the identification of the antigens on RL♂1 and Meth A recognized by CTL and the host immune responses to those antigens. Involvement of regulatory $CD4^+CD25^+$ cells in tumor growth is also described.

The methods for identification of antigen peptides

There are several methods for identifying tumor antigens recognized by CTL. A genetic approach using recombinant cDNA expression libraries has been widely used.[12, 13] Transient transfectants are screened for the ability to stimulate the CTL. Repeated screening while lowering the plasmid number in a panel allows the cloning of the gene encoding the antigen. To define the region encoding the antigenic peptide, deletion mutants are prepared. The synthetic peptides which have higher binding score to the MHC class I molecule are analyzed for sensitization of target cells to CTL lysis. Biochemical purification of peptides from the tumor cells or from the MHC class I molecules of the tumor cells has also been used.[14–16] Peptides derived from tumor cells are fractionated by high-performance liquid chromatography (HPLC) and then each HPLC fraction is tested for the ability to sensitize target cells for lysis by the corresponding CTL. The active fraction is further purified by HPLC and sequenced. Lastly, reverse immunology has been useful. The synthesized candidate peptides, deduced from the consensus anchor motif for antigen-presenting MHC molecules in the proteins known to be overexpressed or mutated in tumor cells, are screened by using T cells.[17, 18] This method is used especially for searching CD8 T cell epitopes in the serologically defined tumor antigen by means of SEREX (serological analysis of antigens by recombinant expression cloning).[19]

Of these methods, expression cloning and peptide elution methods have been preferred to identify T cell-recognized antigens for which no molecular information is available. However, there are some intrinsic difficulties in applying these methods. In expression cloning of cDNA libraries, the number of recombinant plasmids in a pool should be less than 100, possibly even less than 50, when CTL sensitivity is low. Therefore to screen 1×10^5 recombinant plasmids, a minimum 1000 plasmid pools must be screened. The assay detects cytokines such as IFN-γ, TNF-α or GM-CSF released in the culture supernatant by ELISA. The purification of plasmids from large numbers of bacterial pools, their transfection, and the subsequent T cell assays for cytokines are considerable tasks. Similarly, peptide elution is technically demanding and it is practically impossible to obtain a fraction containing a single peptide even after repeated HPLC using various phases.[20] As a consequence, it has proven extremely difficult to resolve the reactive peptide sequence from contaminating peptide signals. However, the antigenic peptide has been obtained from RL♂1 radiation-induced leukemia, as we reported previously.[16] Recently, we improved

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the sensitivity and the efficacy of T cell antigen cloning by using large- and small-scale ELISPOT assays instead of ELISA or bioassays to detect cytokines produced by T cells from cDNA expression libraries (see below).[21] By using this new approach, we succeeded in defining the CTL epitope on methylcholanthrane-induced Meth A sarcoma, as described below.[50]

Tumor antigens recognized by CD8 T cells

Since the first discovery by Boon and colleagues,[12, 13] the list of antigens recognized by T cells on human and also murine tumors is growing.[22, 23] Tumor antigens are classified into several groups (Table 1). The first group consists of cancer/testis (CT) antigens encoded by genes that are silent in normal tissues but expressed in placental trophoblasts and testicular germ cells, e.g., P1A in murine mastocytoma P815[24] and MAGE[25] in human melanomas. The second group consists of differentiation antigens expressed on tumor cells and also, at least in some stage of differentiation, on the normal tissue from which the tumor was derived: e.g., TRP2[26, 27] and tyrosinase.[28] The third group of antigens corresponds to peptides derived from proteins that are mutated in tumor cells, e.g., ERK2 in murine methylcholanthrene-sarcoma CMS5[29] and CDK4[30] in human melanoma. Epitopes produced by unusual events that occurred during transcription, splicing, or translation have also been identified in tumors.[31] Another group of antigens is those derived from oncogenic, endogenous, or exogenous viruses. Virally induced mouse tumors and also spontaneous leukemia LEC[32] or carcinogen-induced colon cancer CT26[33] expressed viral antigens recognized by CTL.

Most murine and human tumor antigens fall into these categories, suggesting the relevance of murine tumor models for studying human cancer antigens.[34] Murine tumor models for which the tumor antigens have been molecularly identified are extremely useful in helping to establish the basis of immunotherapy.[35, 36]

Identification of a peptide recognized by CTL on RL♂1

BALB/c RL♂1 is a radiation-induced leukemia.[10] It is a highly immunogenic tumor for BALB/c mice hybridized with certain mouse strains, in which inocula of RL♂1 cells initially grow, forming a tumor, and then regress.[37] Spleen cells from regressor (BALB/c×C57BL/6)F_1 ($CB6F_1$) mice generated CD8 CTL after *in vitro* stimulation. The CTL recognize RL♂1, but not other radiation leukemias, fibrosarcomas, or blasts from normal lymphoid cells. The findings suggest that there is a unique antigen on RL♂1. Adoptive transfer of CTL to BALB/c *nu/nu* mice protected the recipient mice from subsequent challenge with RL♂1 cells.[38] *In vivo* depletion of CD8 T cells abrogated the rejection, whereas depletion of CD4 T cells had little effect.[39, 40] The finding suggested that the RL♂1 rejection response is mediated by CD8 CTL. We identified a unique tumor antigen peptide, pRL1, that is recognized by CTL on RL♂1.[16]

The materials of less than 5000 daltons eluted with 0.1% trifluoroacetic acid (TFA) from 2.5×10^{11} RL♂1 tumor cells were fractionated by HPLC and each fraction was then tested for the ability to sensitize ^{51}Cr-labeled P815 (H-2^d) cells for lysis by RL♂1-specific CTL. The sensitization ability was observed in two fractions, peak a and peak b, with elution times of 23 and 26 min. After several more HPLC purification procedures, the amino acid sequences of peptides in the active fractions of peaks a and b were analyzed by automated Edman degradation. The octamer peptide IPGLPLSL was identified in peak a, and the decamer peptide SIIPGLPLSL in peak b. Octapeptide was eluted from both affinity-purified L^d-molecule and whole extract from RL♂1, and the decapeptide was eluted predominantly from the whole extract, suggesting that the decamer peptide is the natural precursor peptide of the octamer peptide.[41] Amino acid sequence homology search using the National Biochemical Research Foundation (NBRF) database showed that the sequences of these peptides correspond to the normally untranslated 5′ region of *cakt*. 5′-RACE analysis of the RL♂1 *akt* (*cRLakt)* transcript revealed that the murine leukemia virus (MuLV) long terminal repeat (LTR) was inserted into the first exon (Fig. 1).[42] As a consequence, transcription of the *RLakt* gene seems to be accelerated by the strong promoter activity of the LTR sequence. Northern blot analysis demonstrated higher expression of *akt* mRNA in RL♂1 cells than BALB/c thymocytes.

We raised rabbit antiserum against a synthetic peptide consisting of 16 hydrophilic amino acid residues of the carboxyl terminus of the Akt protein. On western blot analysis, the antiserum reacted with both 59K and 56K molecules in the RL♂1 lysate, but with only 56K molecules in the lysate from BALB/c thymocytes.[42] The expression of the 59K molecules was about ten times that of normal 56K Akt molecules as judged by densitometric analysis. *Rlakt*-transfected NIH/3T3 cells exhibited

Table 1. Murine tumor antigens recognized by CTL

Antigen	Tumor	Peptide	Antigen presenting molecule
Cancer/testis antigens			
P1A	P815 (methylcholanthrene-mastocytoma)	LPYLGWLVF	H-2L^d
Differentiation antigens			
TRP-2	B16 (melanoma)	VYDFFVWL	H-2K^b
Antigens resulting from genetic alteration			
connexin-37	Lewis (3LL) (lung carcinoma)	FEQNTAQP	H-2K^b
DEAD box helicase p68	8101-RE (UV-induced sarcoma)	SNFVFAGI	H-2K^b
Akt (LTR insertion)	RL♂1 (radiation induced leukemia)	IPGLPLSL	H-2L^d
MAPK ERK2	CMS5 (methylcholanthrene-sarcoma)	QYIHSANVL	H-2K^d
methionine reductase	P815 (methylcholanthrene-mastocytoma)	GYCGLRGTGV	H-2K^d
ras		LEEYSAM	H-2K^b
ras		GKEEYSAM	H-2K^b
ras		YKLVVVGAV	H-2K^d
ramp (exon extension)	Meth A (methylcholanthrene-sarcoma)	LGAEAIFRL	H-2D^d
Viral antigens			
gag IAP	LEC (spontaneous leukemia)	RRKGKYTGL	H-2D^k
gag	FBL-3 (Friend leukemia)	CCLCLTVFL	H-2D^b
gp70	CT26 (colon carcinoma)	SPSYVYHQF	H-2L^d

some characteristic features of the transformed phenotype: anchorage-independent growth, and high saturation density, lower serum requirement for growth, and tumorigenicity in nude mice.[43]

Immunogenicity of pRL1a peptide

We investigated the generation of cytotoxicity in BALB/c spleen cells by *in vivo* and *in vitro* sensitization with pRL1a peptide either in the original form or in the form of multiple antigen peptide (MAP). BALB/c mice were injected with 100 μg of pRL1a or 120 μg of pRL1a MAP into the footpad twice at an interval of 2 weeks. Two weeks after sensitization, spleen cells were cultured with pRL1a or pRL1a MAP and cytotoxicity was assayed. No significant cytotoxicity was observed by sensitization with pRL1a peptide, while sensitization with pRL1a MAP did generate cytotoxicity.[35]

Tumor-protective activity in BALB/c mice was investigated. BALB/c mice were immunized with 200 or 400 μg of pRL1a peptide, and with 240 or 480 μg of pRL1a MAP into a footpad twice at an interval of 2 weeks. One to 2 weeks later, 2×10^5 RL♂1 cells were inoculated intradermally into the back of the mice and tumor growth was observed. As shown in Fig. 2, a significant growth-inhibitory effect was observed with pRL1a MAP but not with pRL1a.[35]

The mechanism of immunogenicity by pRL1a MAP was investigated. The *in vitro* sensitization with pRL1a MAP was inhibited by the addition of chloroquine (a blocker of acidification of the endosomal compartment), cycloheximide (a protein synthesis inhibitor), and blefeldin A (an inhibitor of protein trafficking from the ER to the Golgi) to the culture, but not by the addition of inhibitors of lysosomal proteases or proteasome. pRL1a MAP was mostly internalized into the endosomal compartment of dendritic cells, leaked to the cytosol, and degraded, and the pRL1a peptide produced was presented via the MHC class I pathway.[44] The efficient presentation of pRL1a on L^d-molecule on DC could elicit a protective immune responses.

We previously demonstrated that as many as 2×10^6 RL♂1 cells were rejected in $CB6F_1$ mice, but no rejection response occurred in syngeneic BALB/c mice even with inocula of less than 1×10^5 cells.[45] We investigated the generation of pRL1a peptide-specific CTL in spleen cells from RL♂1-bearing low-responder syngeneic BALB/c mice and high-responder semi-allogeneic $CB6F_1$ by H-2L^d/pRL1a tetramer staining. The H-2L^d/pRL1a tetramer binding CD8 T cells were efficiently generated in spleen cells from $CB6F_1$ mice compared to those from BALB/c mice (Fig. 3).[46]

Immune regulation has been shown to be involved in the progressive growth of some murine tumors. North and Bursuker[47] demonstrated that CD4 T cells from BALB/c mice carrying syngeneic methylcholanthrene-induced Meth A sarcoma down-regulated the activation of immune effector cells against the tumor. Similar findings were obtained with other murine tumors. *In vivo* depletion of CD4 T cells on day 6 from tumor-inoculated BALB/c mice caused RL♂1 regression.[45, 48] Thus, overexpression of the RLakt molecule seemed to induce CD4 immunoregulatory cells, which resulted in progressive RL♂1 growth in BALB/c mice. *In vivo* administration of anti-CD25 mAb (PC61) caused regression of RL♂1.[49] The treatment resulted in a reduction in $CD4^+CD25^+$ T cells in peripheral lymphoid tissues. Similar findings were also obtained with five leukemias, a myeloma, and two sarcomas derived from four different inbred mouse strains but not with AKSL2, a spontaneous leukemia or RL♀8, a radiation-induced leukemia (Fig. 4).[49]

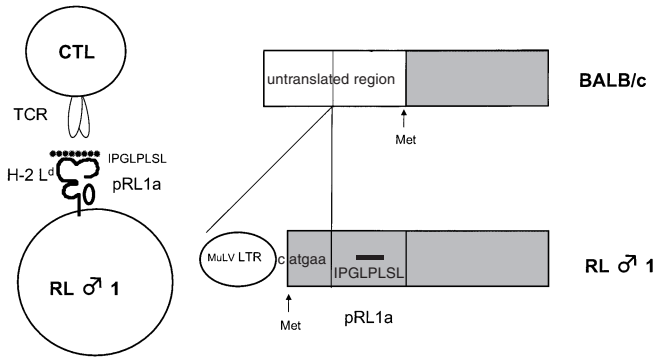

Fig. 1. The structure of the 5′ region of the RL♂1 *c-akt*.[14, 41] MuLV LTR was inserted into the 5′-untranslated region of *c-akt*. Downstream of the LTR, a stretch of six nucleotides of unknown origin was entailed, in which an ATG codon was formed in-frame with the *c-akt* open reading frame. Peptide pRL1a (IPGLPLSL) derived from the untranslated region of c-*akt* was recognized by the RL♂1-specific CTL.

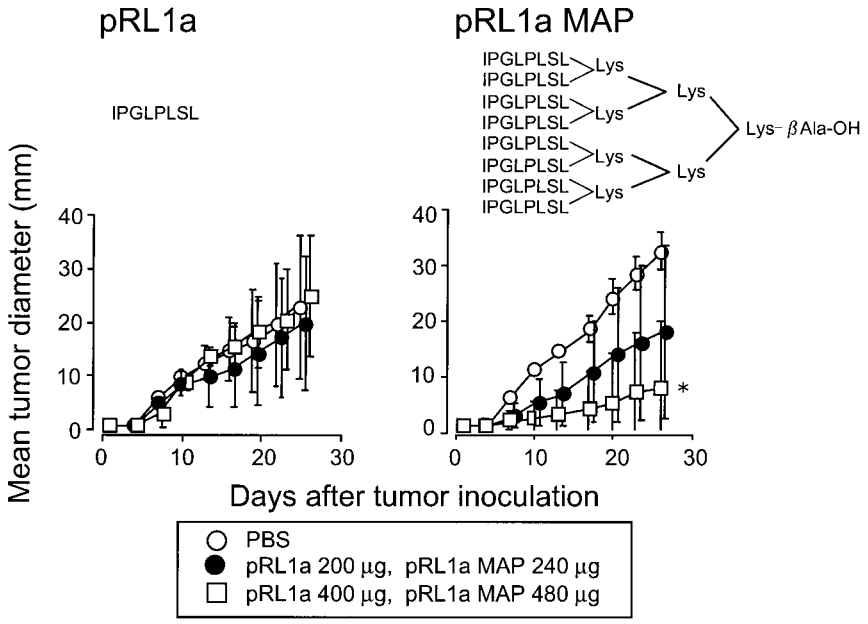

Fig. 2. The effect of sensitization with pRL1a or pRL1a MAP on RL♂1 tumor growth.[33] BALB/c mice were injected twice in the footpad with pRL1a peptide, or pRL1a MAP at an interval of 2 weeks. At 1–2 weeks after sensitization, 2×10^5 RL♂1 cells were inoculated into the backs of the mice. Each experimental group consisted of five mice. Values, mean±SD. * $P<0.01$. Sensitization with pRL1a MAP, but not pRL1a caused significant growth inhibition of RL♂1 tumor.

These findings suggested that $CD4^+CD25^+$ immunoregulatory cells were involved in the growth of the tumors.

Identification of Meth A antigen recognized by CTL

Meth A is a methylcholanthrene-induced sarcoma in BALB/c mouse.[11] The CTL clone AT-1 was derived from Meth A (sv)-rejected $CB6F_1$ spleen cells. AT-1 specifically recognized parental Meth A, Meth A (p), and the Meth A (sv) variant sensitive for CTL lysis in IFN-γ ELISPOT assays. Meth A (sv) but not Meth A (p) was lysed in a standard 4-h ^{51}Cr release assay. Failure of Meth A (p) lysis by CTL appeared to be due to intrinsic resistance to lysis, since lysis by anti-allogeneic H-2^d CTL was also not observed. Meth A antigen specificity of the AT-1 CTL clone was confirmed since none of the BALB/c methylcholanthrene-sarcomas CMS4, CMS5a, CMS5j, CMS8, CMS9 and CMS13, BALB/c radiation leukemia RL♂1, or DBA/2 mastocytoma P815 target was recognized. AT-1 cytotoxicity and IFN-γ ELISPOTs for Meth A (sv) was CD8-dependent and restricted by H-$2D^d$.

Approximately 4×10^5 cDNA clones from Meth A (sv) mRNA were screened by IFN-γ ELISPOT assay according to the ELISPOT method described previously.[21] Following three rounds of screening by large-scale ELISPOT assay and two rounds of screening by small-scale ELISPOT assay, the cDNA clone S35 of 937 bp was obtained.[50] AT-1 CTL were stimulated with S35-transfected CMS5a, CMS8, CMS13 and H-$2D^d$, but not H-$2L^d$ transfected L cells.

To identify the peptide epitope recognized by AT-1 CTL, various truncated S35 mutants were prepared and transfected into CMS8 cells (Fig. 5A). The 3-prime-truncated S35 nt 1–700, but not nt 1–665 or nt 1–350, stimulated AT-1 IFN-γ production. Therefore, we synthesized 9-mer overlapping peptides covering the region A and tested them for stimulation of AT-1 IFN-γ production. However, no stimulation was observed. We then prepared 5-prime-truncated S35 nt 48–700 and nt 443–700 and tested their stimulatory activity, but no stimulation was observed with either mutant. We synthesized 9-mer overlapping peptides covering region B and tested their stimulatory activity. IFN-γ production by AT-1 CTL was observed after stimulation by CMS8 pulsed with the peptide p8 (LGAEAIFRL, S35-peptide) corresponding to nt 32–58. AT-1 CTL lysis of CMS8 pulsed with S35-peptide was detected at 20–30 n*M* peptide concentration. The above results indicated that the downstream sequence spanning nt 665–700 was necessary for the expression of the S35-peptide. A typical splicing donor site at nt 71 and 72 and an acceptor site at nt 699 and 700 were present in S35. CMS8 transfected with S35 mutants with disrupted splicing acceptor sites following introduction of A to C at nt 699 and G to A at nt 701 lost the ability to stimulate AT-1 CTL (Fig. 5A, Exp. III).

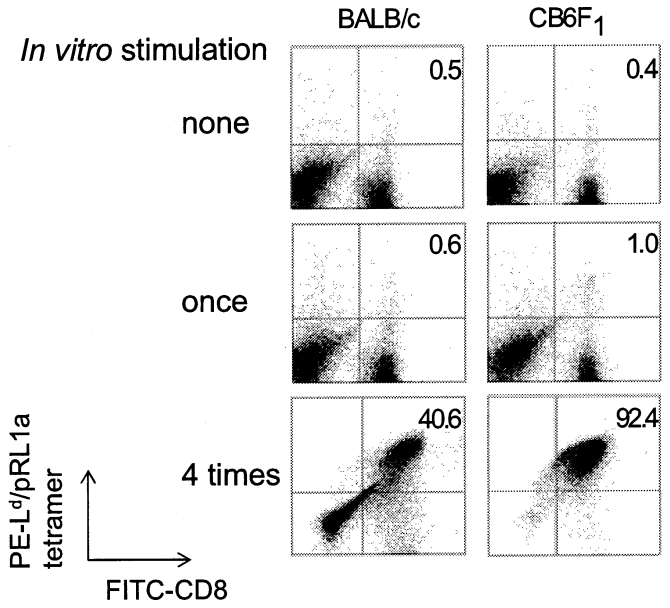

Fig. 3. Flow cytometric analysis of pRL1a-specific CD8 T cells.[45] Spleen cells obtained from RL♂1-bearing BALB/c and $CB6F_1$ mice on day 10 after tumor inoculation were stimulated *in vitro* once and 4 times with MMC-treated RL♂1 cells. Staining of cells with PE-H-$2L^d$/pRL1a tetramer and FITC-CD8 was analyzed with FACScan. The numbers of CD8 T cells stained with H-$2L^d$/pRL1a tetramer were 0.6 and 1.0% in the spleen cells from RL♂1-bearing BALB/c and $CB6F_1$ mice, respectively, after a single *in vitro* stimulation. After 4 *in vitro* stimulations, the numbers of tetramer-positive cells were increased to 41% and 92%, respectively.

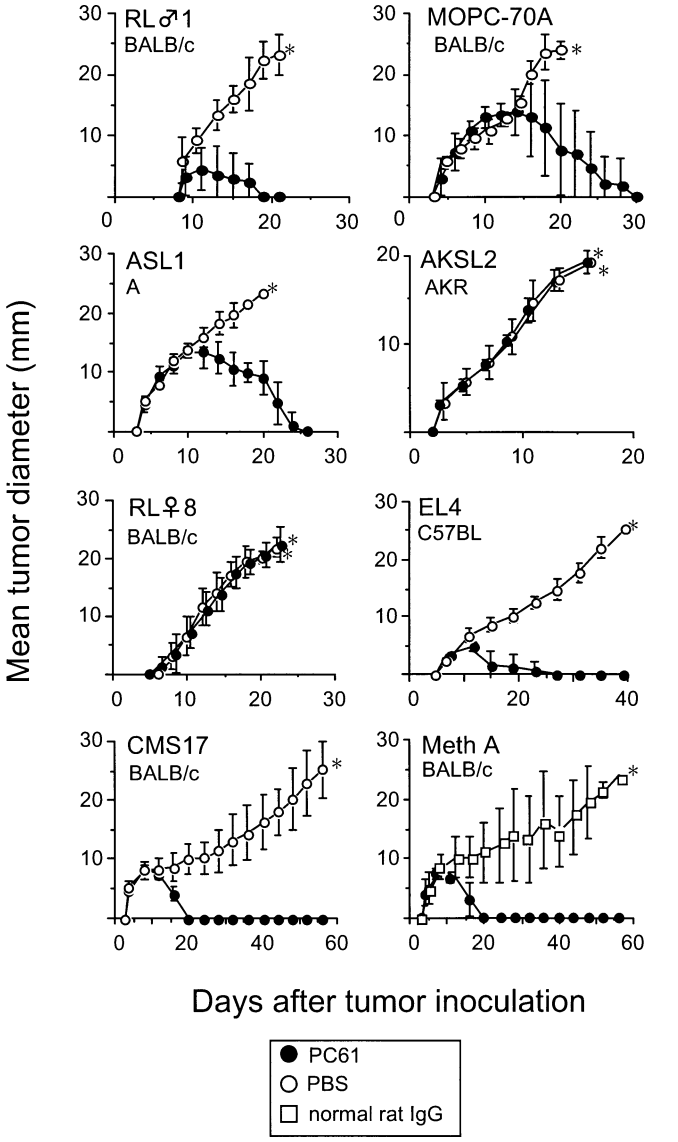

Fig. 4. Effect of administration of anti-CD25 mAb (PC61) on tumor growth.[48] RL♂1 (2×10^5), MOPC-70A (5×10^5), ASL1 (1×10^6), AKSL2 (2×10^5), RL♀8 (2×10^5), EL4 (1×10^5), CMS17 (2×10^6), and Meth A (2×10^5) cells were inoculated into the backs of the mice indicated, PBS, normal rat IgG, or anti-CD25 mAb (PC61) on day–4. A single administration of 0.25 mg of anti-CD25 mAb (PC61) on day–4 caused regression in six of the eight tumors.

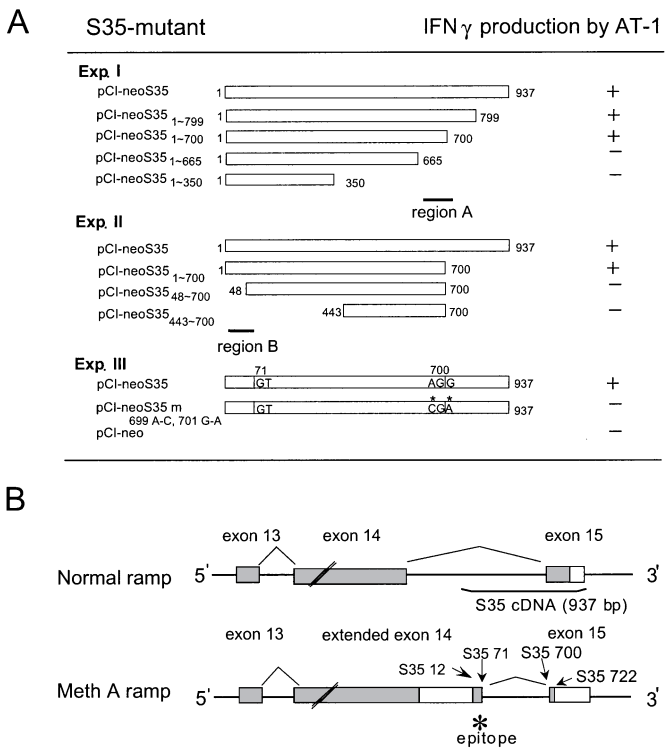

Fig. 5. Localization of the nucleotide sequence in S35 coding for the antigenic peptide and genomic structure of extended exon 14 of Meth A *ramp*.[50] A: The deleted fragments amplified by PCR were cloned in pCIneo vector with *EcoR*I and *Sal*I sites and then transfected into CMS8 cells. IFN-γ production by AT-1 CTL was measured by ELISA. Mutations at nt 699 (A to C) and nt 701 (G to A) in experiment III were introduced by the use of site-directed mutagenesis kits. B: The exons and the introns are represented as boxes and lines, respectively. ORFs are shown in shaded boxes. * shows the location of the antigenic epitope. The nt 12 in the S35 cDNA clone shows the location of the newly created start codon in the extended exon 14. The nucleotide numbers 71 and 700 in S35 represent splicing donor and acceptor sites, respectively, in the Meth A *ramp*.

Analysis of cDNA that coded for the antigen recognized by AT-1 CTL

According to a GenBank BLAST search, nt 700–811 of S35 showed 86% homology to a region of the human retinoic acid-regulated nuclear matrix-associated protein (*ramp*) gene[51], suggesting that S35 is a part of a mouse homolog of *ramp*. From Meth A (p) cDNA, ~2.5 and 3.8 kb RT-PCR products were obtained using human *ramp* nt 124–145 as the 5′-primer and S35 nt 923–937 as the 3′-primer. The nucleotide sequence in the ~2.5 kb PCR product was the same as that in the 3.8 kb PCR product except for lacking nt 2207–3568. The nucleotide sequence and the deduced amino acid sequence of the ~2.5 kb products showed 85% and 89% homology, respectively, to human ramp, spanning exon 1 to 15. Several mouse ESTs with homology to this sequence, but not to nt 2207–3568, were identified in the 3.8 kb product. The selective deletion spanning nt 2207–3568 suggests that this region was an intronic sequence between exon 14 and 15. Therefore, the 3.8 kb PCR product appeared to be derived from an immature mRNA which contain a splicing donor site and an acceptor site for further splicing. These results indicated that *ramp* mRNA with extended exon 14 was expressed in Meth A (Fig. 5B). The newly created ORF from nt 12–722 of S35 contained the epitope sequence.

The *ramp* mRNA expression was examined by RT-PCR. The normally spliced *ramp* mRNA was overexpressed, but the exon 14 extension occurred only rarely in Meth A. The *ramp* mRNA expression was observed strongly in testis and weakly in adrenal gland, esophagus and ovary in normal tissues. Its overexpression was observed in all tumor cell lines examined, including 3 other methylcholanthrene-sarcomas, CMS5a, CMS8 and CMS13, a radiation leukemia RL male 1 and three RadLV-induced leukemias RVA, RVC and RVD, and a mastocytoma P815. No expression of exon 14-extended variant *ramp* was observed in those tumors.

BALB/c mice immunized with the tumor showed resistance to tumor growth, and the immunity was predominantly due to CD8 T cells with help from CD4 T cells. We anticipate that effective induction of protective immune responses against tumor could be achieved through synergism of antigen peptides for CD8 T cells and CD4 T cells. Ono *et al*. showed that the ribosomal protein L11 was the dominant antigen in the humoral immune response against Meth A by serological analysis by SEREX.[52] Matsutake *et al*. then showed that CD4 T cells generated through stimulation with Meth A recognized L11 that was mutated.[53]

Conclusions and perspectives

We have described the identification of CTL epitopes on murine leukemia RL♂1 and sarcoma Meth A. Since these tumors were established in 1963, they have been used in many laboratories for the study of tumor immunology. Both are immunogenic. Our studies revealed CTL recognizing peptide antigens derived from *akt* and *ramp* on RL♂1 and Meth A, respectively. Although CD8 CTL have been shown to be involved predominantly in tumor rejection, CD4 helper and regulatory T cells were also shown to play crucial roles in controlling the outcome for the growing tumor. In this regard, defining CD4 T cell epitopes and analysis of CD4 T cell responses in tumor growth are important. As we described briefly in the case of Meth A, the SEREX approach seems promising for defining CD4 T cell epitopes. Another important issue with regard to tumor antigens is multiplicity and dominance. With RL♂1, pRL1a was apparently the dominant antigen. There were several other less dominant antigens recognized by CTL on RL♂1 which became evident when pRL1a antigen was experimentally diminished. Meth A S35 antigen could be one of the several less dominant antigens. Thus, characterization of all tumor antigens is important to reveal overall host immune response against tumor. Only studies with tumors expressing known T cell epitopes are able to provide us with information relevant to immunotherapy for cancer patients.

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