Source of unique tumour antigens

SIR — there is mounting enthusiasm for the idea that antigens in malignant cells might be used as vaccines to induce tumour-specific cell-mediated immunity (see ref. 1 for review). A fine example of the power of T cells to destroy solid masses of malignant tissue in man has recently been demonstrated by Papadopoulos et al.[2], who transferred mature donor T cells to immunosuppressed recipients of bone marrow grafts in whom Epstein–Barr virus-associated lymphoproliferative disease had developed.

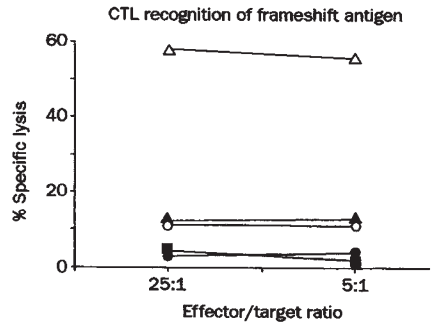
Work has focused on various sources of antigens, including viral[2,3], fetal and tissue-specific antigens[1], and point mutations[4]. Here we suggest an additional, potentially powerful source of tumour antigens that may arise by frameshift mutations. Frameshift mutations give rise to new segments of unique protein. Although these are smaller than most viral proteins, they are more likely than point mutations to contain sequences that will bind to HLA molecules and be presented at the cell surface to cytotoxic T lymphocytes[5].

Frameshift mutations are particularly common in carcinoma of the colon[6,7], which is one of the commonest malignancies in the western world (there are about 160,000 new cases each year in the United States[8]). They also occur in gastric and pancreatic carcinoma[9,10], and occasionally in other malignancies[11]. In carcinomas of the colon the adenomatosis polyposis coli gene (APC) is mutated early in most cases, being detectable in pre-malignant colonic polyps. Nearly all the mutations in APC give rise to a truncation of the encoded protein[6,7]. Approximately half of the mutations are single base changes that result in the formation of a 'stop' codon, and these are not of immunological interest. The other half arise by short deletions or insertions that alter the reading frame[7]. In these cases, beyond the mutation a potentially unique protein sequence will be read off the second or third reading frames until a stop codon is encountered.

We have analysed the series of 43 cases of carcinoma of the colon described in ref. 7. Of these, 21 had frameshift mutations in the APC gene that we calculate would give rise to new sequences of up to 56 amino acids, with a mean length of 16.1 residues. The 11 cases with predicted new sequences of 16 residues or longer are listed in the table. Several of these new reading frames contain regions of sequence that are likely to bind common HLA class I molecules[12], and we have confirmed this for the underlined sequences by demonstrating their ability to induce the assembly of HLA A2 class I molecules *in vitro*[13] (data not shown).

As the mutations tend to cluster[7], certain new reading frames are represented more frequently than others. To see whether these sequences could be immunogenic, we cloned the most commonly expressed new reading frame, expressed it in recombinant vaccinia, and vaccinated BALB/c mice. These animals produced a vigorous cytotoxic T-lymphocyte (CTL) response to the sequence in bold type, which was antigen-specific and MHC (K^d) restricted (see figure). This result demonstrates the viability of using these sequences as immunogens.

Much work is required to establish the value of these sequences as tumour vaccines. Although the 53-amino-acid fragment containing this epitope was apparently correctly processed in L cells (see figure), and the colonic tumour-derived cell line LoVo (data not shown) after infection with recombinant vaccinia, it is not known whether processing of the full-length protein would occur in malignant colonic epithelial cells *in vivo*. In addition, such cells may be highly mutable[14], and may lose the function of genes required for antigen presentation[15,16]. In summary, frameshift mutations offer an additional source of tumour-specific antigens that are potentially more immunogenic than point mutations, but less so than viral antigens. They are relatively easy to detect, are common in carcinoma of the colon and other gastrointestinal malignancies, and should give rise to protein fragments that are likely to bind to the common HLA class I molecules in a significant proportion of cases.



Recognition of a frameshift antigen by K^d-restricted CTL. BALB/c mice were primed with 10^7 PFU recombinant vaccinia virus (M-53-Q-VAC) encoding a 53-amino-acid sequence (MAPVIFQIALDKPCHQAEVKHLHHLLKQLKPSEK YLKIKHLLKRERVDLSKLQ) from the second reading frame of human APC containing the sequence KYLKIKHLL (in bold type in the table). Spleen cells were restimulated and maintained *in vitro* with the peptide KYLKIKHLL derived from this reading frame, and tested in a standard ^{51}Cr release assay at the effector target ratios shown on L/Db target cells[5]. Target cells were either uninfected (solid squares); infected with M-53-Q-VAC (solid circles); infected with K^d-VAC alone (solid triangles); infected with M-53-Q-VAC and K^d-VAC (to provide the class I restriction element) (empty triangles); infected with K^d-VAC and Ub-R-NP-VAC (encoding a short lived influenza nucleoprotein as a control) (empty circles). K^d-VAC was kindly provided by J. Yewdell.

NEW SEQUENCES GENERATED BY FRAMESHIFT MUTATIONS IN THE APC GENE

Codon	Mutation	New Sequence	Size
298	2bp del	SSST/LCTSKADKSSGNQGGNGVFIVVNAWYS	27aa
540	1bp del	SEDL/TAGYCKCFEEFVLASRCK	18aa
1068	4bp del	EQRQ/GIKVQLILFILRALMINTSSSNHIL DSRNVFLHTGHGEPMVQKQIEWVLIMELIKM	56aa
1353	8bp del	HKAV/FRSEISLQKWCSDTQKST	18aa
1398	1bp del	DSFE/SVRLPAPFRVNHAVEW	16aa
1420	1bp del	IISP/VIFQIALDKPCHQAEVKHLHHLLK QLKPSE**KYLKIKHLL**LKRERVDLSKLQ	51aa
1439	1bp del	RSKT/LHHLLKQLKPSE**KYLKIKHLL** LKRERVDLSKLQ	33aa
1446	10bp del	PPQT/GE**KYLKIKHLL**LKRERVDLSKLQ	23aa
1488	1bp del	DADT/YYILPRKVLQMDFLVHPA	18aa
1490	1bp del	DTLL/LLPRKVLQMDFLVHPA	16aa
1493	11bp del	LHFA/SRWIFLFIQPECSEPR	16aa

The data are derived from ref. 7. The sequences underlined were found to stabilize the assembly of HLA A2 as described[13], the sequence in bold induced K^d restricted CTL in BALB/c mice.

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