THE CARCINOEMBRYONIC ANTIGEN GENE FAMILY

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Proceedings of the International Conference on the CEA Gene Family, Sapporo, 15 October, 1988

Edited by
Akira Yachi, M.D.
Professor & Chief, Department of Internal Medicine (Section 1), Sapporo Medical College, Sapporo, Japan

John E. Shively, Ph.D.
Chairman, Division of Immunology, National Medical Research Institute of the City of Good Hope, Duarte, California, USA

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Preface

This book reflects the need to update our current knowledge of the CEA gene family. Since CEA was discovered in 1965, it has become the most intensely studied tumor marker for colon cancer. Although it has long been clear that CEA is one member of a gene family consisting of a number of closely related cell surface glycoproteins, the exact details of their interrelationships has been slow to emerge because of the difficulties in purifying and characterizing these heterogeneous glycoproteins which are usually present in complex mixtures. The situation was significantly improved during the last five years by analysing these antigens with monoclonal antibodies, but structural details were still lacking. The molecular cloning of the genes has changed the situation dramatically. In the last two years, there has literally been an explosion of information regarding the CEA gene family.

In order to promote the rapid dissemination of this new information, a meeting was held in Sapporo, Japan, in October 1988. Most of the research groups which have been highly involved in the molecular cloning of the CEA genes were represented, and presented their latest findings. In addition, updates were presented on protein and carbohydrate structural studies on CEA, together with the latest analysis of its antigenic determinants by synthetic peptide and anti-idiotypic analysis.

Perhaps the most exciting aspect of this meeting, summarized in this book, are the extent of the CEA gene family and its relationship to the immunoglobulin gene superfamily. There are at least twelve members of the gene family, all presumably located on chromosome 19. The members include NCA (non-specific crossreacting antigen), the smallest and perhaps archetypal member of the family, which is found in lung epithelial cells and granulocytes, and usually expressed in colonic tumors along with CEA. BGPI (biliary glycoprotein I) was originally found in bile ducts, but is now known to be widely distributed on epithelial cells, and is unique in having several transmembrane forms. Pregnancy-specific β_1-glycoproteins (PSβG) represent previously unsuspected members of the CEA gene family, and are found in fetal liver and placenta, and can be found in high levels in pregnant serum. CEA is the largest member of the gene family and is primarily located in colon, with highest levels found in fetal colon and adenocarcinomas of the colon.

The CEA gene family is a distinct subgroup within the immunoglobulin gene family. The Ig-family includes immunoglobulins, major histocompatibility antigens, T-cell receptors, Fc and poly-Ig receptors, neural cellular adhesion molecule (N-CAM), and a variety of other proteins. The common theme for these proteins is cell-cell or cell-ligand interactions. Do the members of the CEA gene family have similar functions? The first example of functional studies on CEA is presented here, suggesting that CEA may function as a cell-cell adhesion molecule. However, the molecular details of the interaction have yet to be studied. The situation

becomes even more interesting when one considers that CEA and NCA are anchored to cell membranes via a glycosylphosphatidylinositol diacylglycerol linkage, a linkage only recently discovered. Cell surface proteins linked to membranes via this linkage can be reieased by a specific phospholipase, which may simultaneously transmit an intracellular message via the released diacylglycerol moiety. The molecular signals and mechanism for this release have not been dissected yet for CEA or NCA, but are likely to be involved in function.

The evolutionary aspects of the CEA gene family are also under investigation. Mouse and rat genes have been cloned which correspond to various members of the family. Mouse CEA appears to be analogous to human CEA in terms of sequence and domain arrangement, but the data on rat CEA suggests a different type of domain arrangement. The overall sequence homology of the mouse and rat genes to the human CEA is no more than 50%, suggesting a rapid divergence. The evolution of the more complex members of the family from NCA seems likely. The most distinguishing feature of the gene family is the 107 amino acid N-terminal domain which has no cysteine residues (unlike most members of the Ig family) and bears homology to the variable region of immunoglobulin, including conservation of a number of key amino acid residues forming a salt bridge between β-sheet structure.

Thus, through a combination of new approaches and findings our knowledge of the structure and function of the CEA gene family has substantially grown in the last two years. Most importantly, we can now place this family of glycoproteins within the context of other genes and normal cell function. This information, in turn, should aid in our understanding of the role of CEA in cancer. There remains an enormous amount of work to be done, but the critical groundwork is now in place. This book will help interested researchers keep abreast of the latest developments.

J.E. Shively
Duarte, CA

Opening Remarks

Dr Akira Yachi's Opening Remarks to the International Congress on the CEA Gene Family, October 15, 1988, Sapporo, Japan

It is a great pleasure to extend a cordial welcome to all of you on behalf of the Organizing Committee of this International Conference on the CEA Gene Family.

As you know, the recent advancement of studies on the CEA gene family has succeeded in identifying the precise structures of CEA, NCA, BGPI and pregnancy-specific β-glycoprotein (PSβG). Some other members of the CEA gene family have been or will be isolated and identified. The sugar chains of CEA and the related antigens have also been identified.

Furthermore, it is now possible to prepare monoclonal antibodies to synthetic peptides of CEA and its related antigens, which are truly specific for a given antigen. cDNA probes are now available to detect and identify mRNAs for each CEA gene family. Therefore, I believe that it is now particularly important for investigators to present each observation, to freely exchange their ideas and to discuss the structure, function and use of these molecules for clinical studies.

Fortunately, our study group on the immunological and molecular nature of CEA and the other tumor antigens has been supported by a grant in-aid from The Japanese Ministry of Education, Science and Culture. Last year when I visited Dr Shively, he earnestly suggested I design such a compact congress on the CEA gene family in Sapporo. Therefore, my colleagues and I have planned to have a kind of joint meeting. It is our great honour and pleasure to invite seven distinguished scientists from outside of Japan who have contributed remarkably to the development of this field of study. We acknowledge the support of The Ministry of Education, Science and Culture, Japan and Suntory Institute for Biomedical Research.

I hope that you will enjoy your discussions with each other, mutually exchange opinions and deepen friendships, so as to make this Conference a real success. Thank you very much.

Akira Yachi, M.D.
Chairman of the Conference,
Sapporo Medical College

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[1] Department of Internal Medicine (Section 1), Sapporo Medical College, S-1, W-16, Chuo-ku, Sapporo 060 and [2] Laboratory of Molecular Biology, Suntory Institute for Biomedical Research, Osaka 618, Japan

Introduction

Carcinoembryonic antigen (CEA) has been used in clinical medicine as one of the most popular tumor markers ever since Gold [1] found it in colonic carcinoma and fetal colonic tissues. The level of circulating CEA in the sera of cancer patients changes in association with tumor progression, with recurrences after operation and with metastasis. Numerous studies [2–5] demonstrated that CEA is a 180–200 kDa glycoprotein and expressed not only in colonic adenocarcinoma, but also in other adenocarcinomas, such as carcinomas of the stomach, pancreas, bile duct, lung, breast, ovary and so on. Furthermore, CEA is also expressed in normal epithelial tissues, as well as malignant transforming tissues, although the amount of CEA has been considered to be low [6,7].

Immunological and biochemical studies have shown that there are many CEA-related antigens that have a cross-reactivity to CEA, including non-specific cross-reacting antigen (NCA) [8], tumor-extracted CEA-related antigen (TEX) [9], normal fecal antigen (NFA) [10], meconium antigen (MA) [11] and biliary glycoprotein I (BGPI) [12]. Recently, gene cloning of CEA [13–15], NCA [16,17], BGPI [18] and pregnancy-specific β-glycoprotein (PSβG) [19,20] has been successfully performed and the structures of these antigens have been determined.

Interestingly, these CEA-related antigens have shown similar domain structures and a high homology in their amino acid sequences. Studies on gene cloning have also indicated that CEA and NCA belong to members of the immunoglobulin supergene family.

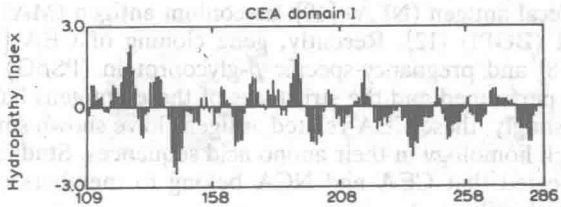
These molecular cloning results have suggested that it is very difficult to prepare monoclonal antibodies specific for a given antigen of the CEA family if one uses ordinary antigens, such as soluble CEA, or cell extracts from cultured cells, such as an immunogen, since the amino acid sequences are very similar among CEA family antigens. Therefore, we have decided to use synthetic peptides as immunogens in order to generate specific monoclonal antibodies to epitopes which are unique for a given antigen. This study will further clarify the relationship between CEA and other family antigens from the molecular aspects and therefore it will be possible to compare the DNA and mRNA levels of CEA and other family antigens with the products of these genes in cancerous tissues as well as in premalignant tissues.

Synthesis and characterization of CEA peptide

According to the amino acid sequences of CEA determined by gene cloning studies, 22 amino acids (peptide P1) from 119 to 140 in domain I of CEA were synthesized by an amino acid synthesizer and then tested for amino acid composition by HPLC in order to confirm the data. The result is shown in Table 1. This synthetic CEA peptide P1 was selected as an immunogen firstly because of its hydrophilic character and secondly because of its common presence in sequences of domains I, II and III of CEA (Fig. 1 and Table 1).

Development of monoclonal antibodies to synthetic CEA peptide (P1)

Intraperitoneal immunization of synthetic peptide, P1 (100 μg), which was suspended in Freund's adjuvant was done three times on BALB/c mice, after which cell fusion was performed according to the standard procedure. Supernatants of hybridomas were tested for their reactivities with peptide P1 and with native CEA which was extracted from colonic tumor cells. Briefly, microtiter plates coated with P1 or CEA were incubated with supernatants for 5 h and, following washing, they were incubated with ^{125}I-labelled goat anti-mouse IgG Fc portion as a tracer. As shown in Table 2, the resulting monoclonal antibodies (MoAbs) were divided into two groups: group 1 (MoAb P1-706) reacted with synthetic peptide P1, but not with native CEA preparation, while group 2 (MoAb P1-234 and MoAb P1-255) reacted with either type of antigen. These MoAbs did not react with synthetic CEA peptide P3, P4, P5 or P6. To confirm this serologic profile of anti-CEA peptide MoAbs, an immunoblotting assay was performed, using purified native CEA as the antigen. Anti-CEA peptide MoAb P1-234 and P1-255 had reactivity to native CEA (about 200 kDa) in immunoblotting, while MoAb P1-706 did not (Fig. 2).



	119																				140
Domain I	N	S	K	P	V	E	D	K	D	A	V	A	F	T	C	E	P	E	T	Q	D	A
	297																				318
Domain II	N	S	N	P	V	E	D	E	D	A	V	A	L	T	C	E	P	E	I	Q	N	I
	475																				496
Domain III	N	S	K	P	V	E	D	K	D	A	V	A	F	T	C	E	P	E	A	Q	N	I

FIG 1 Analysis of surface area of CEA on peptide sequence from hydropathy index.

TABLE 1 Composition analysis of amino acids in P1 peptide

	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Trp	Lys
Theoretical	3	3	1	—	—	—	2	3	2	—	2	—	—	—	1	—	2
Experimental	3	3	1	—	—	—	2	3	2	—	2	—	—	—	1	—	2

TABLE 2 Reactivity of anti-CEA peptide monoclonal antibodies with various preparations of CEA peptides

	Amino acid sequences	Monoclonal antibodies		
		P1-706 (cpm)	P1-234 (cpm)	P1-255 (cpm)
Synthetic peptide P1	119–140	21445	26628	23143
P3	202–221	1587	1164	1080
P4	367–386	1439	1004	1216
P5	558–577	1665	1022	934
P6	1–20	1258	864	832
Native CEA	1–668 + CHO	2694	25549	23788
Native NCA	1–310 + CHO	2320	18107	16858
Bovine serum albumin		1591	1775	852

Characterization of anti-CEA peptide MoAbs

Reactivity to recombinant CEA domains

The reactivities of anti-CEA peptide MoAbs were tested for binding to four kinds of recombinant domain peptides of CEA, which were generously gifted by Dr Nakazato and Dr Oikawa of Suntory Institute for Biomedical Research. Recombinant domains N, I, II and III of CEA were amino acid sequences of 1–106, 107–262, 301–478 and 479–668, respectively. A CEA domain-coated plate was incubated with ^{125}I-labelled anti-CEA peptide MoAbs for 3 h at 4°C, and following 5 washings, bound cpm was counted in a γ-counter. Table 3 showed that MoAb P1-706 reacted with only domain I, MoAb P1-234 reacted with domain I and III and MoAb P1-255 reacted with domain I, II and III.

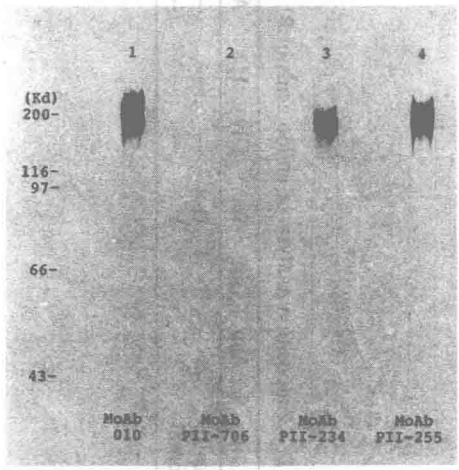


FIG 2 Immunoblotting of CEA reacted with anti-CEA peptide MoAb. Lanes 1, 2, 3, 4: non-reduced condition. Lane 1: MoAb 010 specific to native CEA. Lanes 2, 3, 4: MoAb P1-706, P1-234, P1-255 specific to CEA peptides.

TABLE 3 Reactivity of anti-CEA peptide MoAbs with CEA domains

	Amino acid sequences	^{125}I-labelled anti-CEA peptide MoAb		
		P1-706 (cpm)	P1-234 (cpm)	P1-255 (cpm)
CEA domain N	1–106	1910	1226	1487
I	107–262	4573	11 710	12 505
II	301–478	1941	1413	13 689
III	479–668	1876	11 980	13 969
P1 peptide	119–140	9940	20 883	14 176
native CEA	1–668 + CHO	1175	5812	16 849
negative control		1264	1100	1844

TABLE 4 Reactivity pattern of conventional anti-CEA monoclonal antibodies with various preparations of CEA peptides

	Amino acid sequences	^{125}I-labelled conventional anti-CEA MoAb			
		group 1 MA208 (cpm)	group 2 CEA281 (cpm)	group 3 12/140/7 (cpm)	group 4 12/140/1 (cpm)
Synthetic peptide P1	119–140	459	516	378	390
P3	202–221	419	513	355	387
P4	367–386	419	515	327	330
P5	558–577	484	420	368	351
P6	1–20	471	460	294	354
Native CEA	1–668 + CHO	60 416	6065	19 157	16 943
BSA		297	316	266	235

Comparison of anti-CEA peptide MoAbs with conventional MoAbs

An international workshop on the epitope reactivity of MoAbs against CEA has summarized that conventional anti-CEA MoAbs have been divided into several groups according to serological studies, mainly inhibition assay. In our study representative MoAbs of each group were tested for reactivity to purified native CEA, NCA and synthetic peptides. None of them reacted with synthetic peptides, while all reacted with native CEA, suggesting that the epitopes recognized with these conventional anti-CEA MoAbs do not exist either in P1, P3, P4, P5 or P6 (Table 4). In other words, anti-CEA peptide MoAbs established in our laboratory appear to be different from the conventional ones and recognize epitopes with which conventional MoAbs do not react.

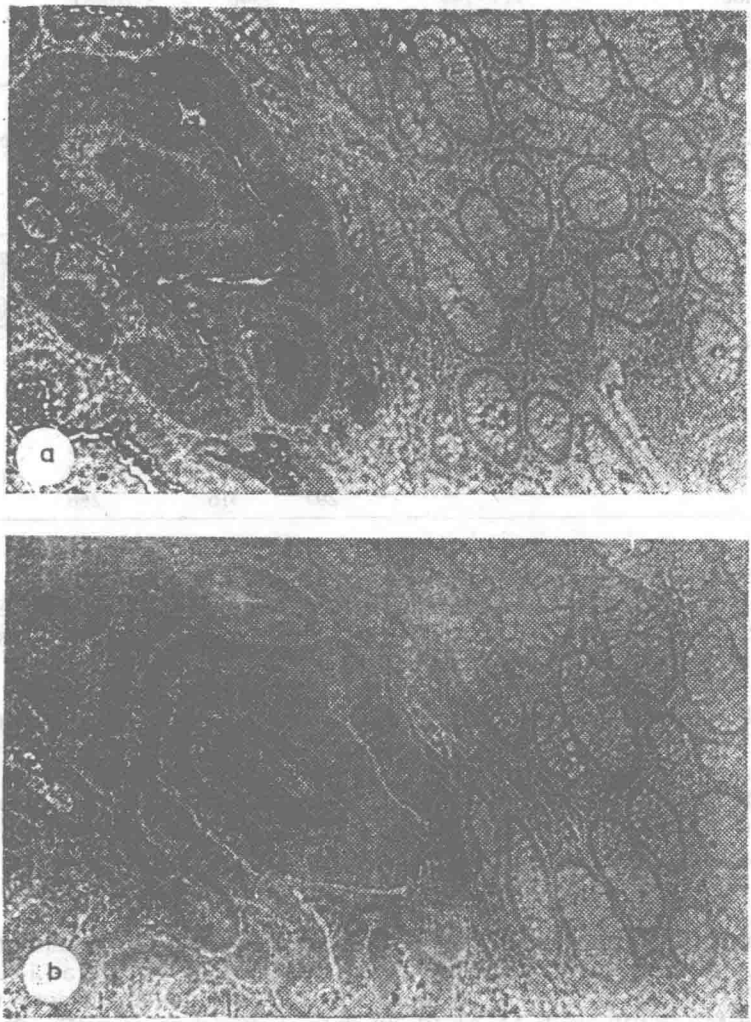
Staining pattern in tissues by immunoperoxidase method

Immunostaining in colonic and gastric carcinoma tissues was carried out, utilizing anti-CEA peptide MoAbs and conventional anti-CEA MoAb 010 as a control. MoAb P1-234 and MoAb P1-255 showed a similar distribution in carcinoma tissues to that of MoAb 010, while MoAb P1-706 did not stain the tissues (Fig. 3).

Characterization of the epitope recognized by MoAb P1-706

Anti-CEA peptide MoAb P1-706 reacted with CEA domain I, but not with domains N, II, III or native CEA.

In order to see the steric hindrance of the epitope due to carbohydrate moiety of CEA, further examination was carried out utilizing periodate-treated CEA in an immunoblotting assay. As shown in Fig. 4, MoAb P1-706 had a reactivity with CEA which was treated with 1% periodate for 30 min, but not with non-treated CEA, while MoAb P1-255 reacted with both of them. This result suggests that the reactivity of MoAb P1-706 with native CEA was affected by the existence of carbohydrate moiety.



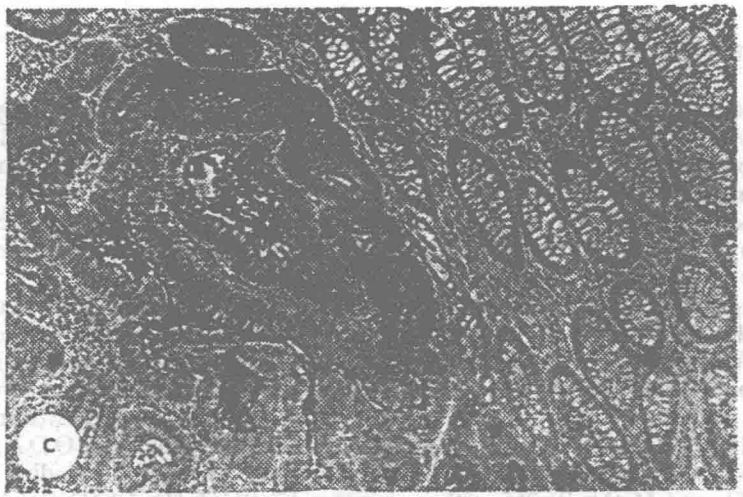


FIG 3 Immunoperoxidase staining of colonic carcinoma tissue with anti-CEA and anti-CEA peptide MoAbs. (a) Anti-CEA MoAb 010. (b) Anti-CEA peptide MoAb P1-706. (c) Anti-CEA peptide MoAb P1-255.

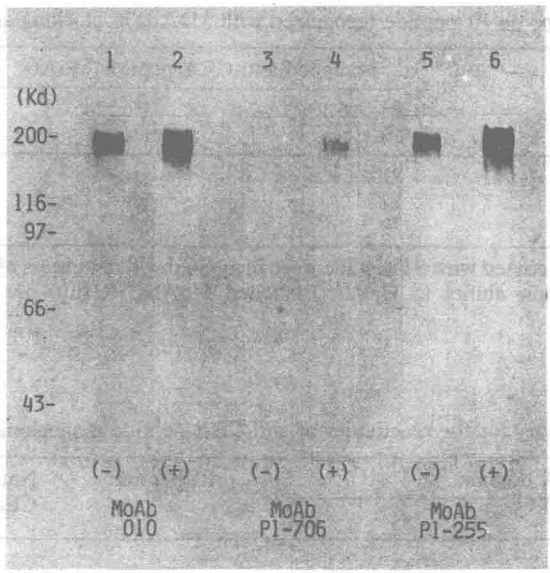


FIG 4 Immunoblotting of periodate-treated CEA reacted with anti-CEA peptide MoAb. Lanes 1, 3, 5: (−) non-treated CEA. Lanes 2, 4, 6: (+) periodate-treated CEA.

Possible amino acid sequences recognized with anti-CEA peptide MoAbs

In order to analyse the spatial relationship among epitopes on synthetic peptide P1 recognized with anti-CEA peptide MoAbs, a blocking assay was performed. MoAb P1-706 and P1-234 did not inhibit each others binding to peptide P1, but when each was paired with P1-255 inhibition occurred (Table 5). This result suggests that the epitope recognized with MoAb P1-255 was spatially close to that recognized with MoAb P1-706 and to that with MoAb P1-234, but the epitopes recognized with MoAb P1-706 and P1-234 were far from each other.

The different reactivities of the MoAbs with native CEA, NCA and CEA domains N, I, II and III allowed us to further analyse possible amino acid sequences in peptide P1 recognized by each MoAb. Based on all the results summarized in Table 6, computer analysis was done to determine their possible epitopes. The possible amino acid sequences as epitopes recognized with MoAbs are suggested in Fig. 5. Namely, MoAb P1-706, a sequence between 119–140 including TQDA, MoAb P1-234, a sequence between PVEDKDAVAFTCEPE (excluding F3, F4 and F5), and MoAb P1-255, either sequence F3 (PVED), F4 (DAVA) or F5 (TCEPE).

In addition to this result, the blocking assay suggests that the more possible candidates as epitopes are PETQDA for MoAb P1-706, PVEDK for MoAb P1-234 and DAVA or TCEPE for MoAb P1-255.

TABLE 5 Epitope on P1 peptide recognized with MoAbs in blocking assay[a]

Cold inhibitor	^{125}I-labelled anti-CEA peptide MoAbs		
	P1-706 (%)	P1-234 (%)	P1-255 (%)
MoAb P1-706	81	6	53
MoAb P1-234	12	85	41
MoAb P1-255	79	87	90

[a] Microtiter plates coated with P1 peptide were incubated with an excess of cold MoAbs and then tested for their ability to bind ^{125}I-labelled MoAbs. Results are expressed as % blocking.

TABLE 6 Summary for the reactivities of anti-CEA peptide monoclonal antibodies

MoAb	CEA domain				P1 peptide	Native CEA	Native NCA
	N	I	II	III			
P1-706	–	+	–	–	+	–	–
P1-234	–	+	–	+	+	+	+
P1-255	–	+	+	+	+	+	+

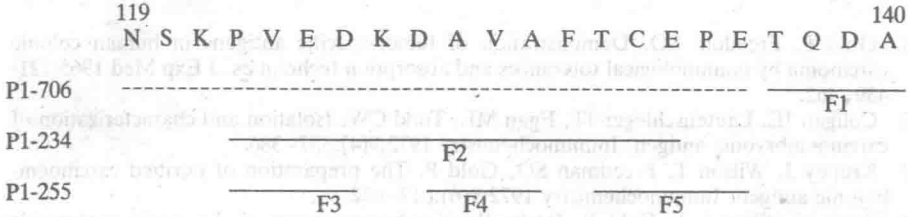


FIG 5 Possible sequences of amino acids of CEA recognized by anti-CEA peptide monoclonal antibodies.

Comments

The quality and direction of CEA research have clearly changed since the amino acid sequences of CEA were determined by gene cloning studies [13–20] in 1987 and 1988.

It was proposed that CEA and NCA were members of the immunoglobulin supergene family [17]. This hypothesis suggested that CEA and NCA might be derived from a primitive immunoglobulin-like gene. These results stimulate our interest in the characters of the CEA family in association with their biological functions and their evolutionary relationship.

We have developed and characterized monoclonal antibodies to synthetic CEA peptide. The results might suggest that: **(1)** anti-CEA peptide MoAbs recognize distinct epitopes to conventional anti-CEA MoAbs, since conventional MoAbs do not react with peptide P1; **(2)** epitopes are composed of about 4–6 amino acids; and **(3)** carbohydrate moiety on CEA affects the binding of the anti-CEA peptide MoAb P1-706 to peptide P1.

This approach will eventually allow us: **(1)** to analyse the tertiary or fine structure of CEA; **(2)** to see the influence of the carbohydrate moiety on CEA peptide bone structure; **(3)** to develop a new type of MoAb which cannot be generated with a conventional immunization method; **(4)** to establish more useful assays for detecting CEA in the sera of patients and to investigate epitope distribution in carcinoma tissue with truly specific MoAbs for each CEA family antigen; **(5)** to develop anti-idiotypic antibodies against anti-CEA peptide MoAbs; **(6)** to study the internal image of determined amino acid sequences mimicked by anti-idiotypic antibody; and **(7)** to determine a peptide which would be more effective for vaccination by selecting the anti-idiotypic antibody to modulate immune response in hosts.

Acknowledgements

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