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Human Lymphocyte Response to CEA: Titration of Different CEA Samples and Neutralization Experiments with Monoclonal Anti-CEA Antibodies

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Abstract—*By means of the macrophage electrophoretic mobility technique a striking digestive system cancer-associated human lymphocyte response to CEA has been found during a large-scale study including tests in 499 individuals. The question to be answered by this study was whether this response is really CEA-specific. Titration experiments with 3 different CEA preparations in lymphocytes from 5 colorectal cancer patients showed that the threshold dose of CEA necessary to induce lymphocyte responses amounts to 50–100 ng CEA per ml and 10^6 lymphocytes, regardless of the CEA origin and its state of purity. The CEA specificity of the responses was proved by neutralization experiments with 3 CEA-specific monoclonal antibodies. When allowed to react with CEA before lymphocyte incubation, the MABs prevented CEA from inducing lymphocyte responses. Appropriate murine control myeloma protein did not influence these responses. The reactivity of these lymphocyte samples to a teratocarcinoma extract could not be prevented by treating this material with CEA-specific MABs before incubation. Preliminary attempts to enrich the lymphokine(s) released after CEA stimulation resulted in recovery of the activity within 2 arbitrarily cut Sephadex G-100 fractions comprising the mol. wt range of 3000–47,000.*

INTRODUCTION

CEA IS not commonly regarded as being immunogenic in man. This view is based both on the failure of CEA to induce blastogenic responses in lymphocyte cultures from patients with gastrointestinal cancer and from pregnant women [1] as well as on unsuccessful attempts to detect CEA-specific antibodies in human sera [2, 3]. The antibody findings by Gold [4] and Gold *et al.* [5] conflict with data published by von Kleist and Burtin [2] and by LoGerfo *et al.* [3], the results of which have seriously challenged the CEA specificity of the antibodies detected in human sera by Gold's group. As we have published previously, human lymphocytes can be shown to respond to CEA by means of the macrophage electrophoretic mobility technique. The response frequencies among different patient groups exhibit striking digestive system cancer-associated patterns [6, 7]. To date, approximately 500 individuals have been tested. The data obtained are listed in Table 1.

The MEM technique detects lymphokine(s) released by lymphocytes upon mitogenic stimulation [8, 9] or antigenic restimulation *in vitro* [10, 11]. This technique is suitable to reveal concomitant sensitization of lymphocytes to fetal and tumour-associated antigens during pregnancy or tumour growth [12–14]. When allowed to act on guinea pig macrophages, these lymphokines induce an immediate hyperpolarization of the cell membrane which is followed by a depolarization [15, 16]. The negative charge density of the macrophage membrane decreases shortly after incubation with these lymphokines

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Abbreviations: CEA = carcinoembryonic antigen; MEM = macrophage electrophoretic mobility; RIA = radioimmunoassay; EDTA = ethylene diaminetetra-acetic acid; PBS = phosphate-buffered saline; PPD = purified protein derivative; MAB = monoclonal antibody; Ca = carcinoma.

Table 1. Reactivity to CEA in 499 individuals as detected by the MEM technique (arbitrary cut-off level: macrophage slowing ≥ 5%)

Diagnosis	No. of responders / No. of individuals tested	%
Colorectal carcinoma (including 4 anal Ca)	99/106 (4/4)	93
Pancreatic carcinoma	5/5	100
Stomach carcinoma	34/70	48
Breast cancer	2/27	7
Lung cancer	0/19	0
Kidney cancer (hypernephroma)	3/26	11
Carcinoma of larynx or tongue	5/26	19
Soft tissue or osteogenic sarcoma	0/9	0
Other solid malignant tumours (including 4 teratocarcinomas)	6/21 (2/4)	28
Inflammatory diseases and benign proliferative disorders of the digestive system (including 12 colorectal adenomatous polyps)	12/58 (4/12)	20
Pleomorphic adenomas of the parotid gland	0/8	0
Pregnant women	4/17	23
Healthy controls and patients with metabolic and degenerative diseases not listed above	7/107	6

and remains reduced for at least 120 min [16], during which time this particular lymphokine effect can be measured by means of the cell electrophoretic technique.

Considering all the data available, it is beyond any doubt that biologically active lymphocyte products can be detected using the MEM technique. However, the open question with regard to the CEA-directed responses described is whether human lymphocytes respond to CEA itself or eventually react with minor contaminants co-purified with CEA. Recently we have shown that conventional anti-CEA antisera can specifically neutralize the active components of a CEA preparation, thus preventing lymphocyte responses [17].

But even these experiments, of course, cannot completely exclude the possibility of minor CEA-associated impurities being reponsible for the lymphocyte responses in our system.

This report is devoted to a series of experiments which are to settle this open question.

The first part of this paper details investigations, the results of which show that 50–100 ng CEA, regardless of its origin or state of purity, cause lymphocytes from colorectal cancer patients to respond. The second part deals with neutralization studies using 3 CEA-specific monoclonal antibodies which prevent CEA from inducing human lymphocyte responses. Finally, it will be shown that the specific lymphocyte products released upon CEA contact can be enriched by Sephadex chromatography within a mol. wt region of 3000–47,000.

MATERIALS AND METHODS

Antigens

Several CEA batches have been prepared by means of 0.6 M perchloric acid extraction and, subsequently, by several column chromatography steps, including ion exchange chromatography [18, 19]. The standard CEA–anti-CEA system has been adjusted to the Hoffmann-La Roche RIA kit [19].

The following preparations have been used: CEA 5/II (Standard Dresden) from liver metastases of a primary colon carcinoma (218,500 ng CEA per mg dry substance), and CEA 2-34 from a primary colon carcinoma (400 ng CEA per mg dry wt). CEA 11 resulted from pre-purified CEA batches of primary colon cancer tissues and liver metastases which had been pooled and further purified by DEAE Sepharose chromatography (72,000 ng CEA per mg dry substance). The First British Standard for CEA (approximately 4703 ng CEA per mg dry substance, including the added lactose, according to Laurence *et al.* [20]) was kindly provided by the National Institute for Biological Standards and Control, Holly Hill, London. For our preparations liver metastases and liver tissue were obtained at necropsy 12 hr *post mortem*; primary cancers were prepared from

surgically removed organs within a few hours after operation.

The teratocarcinoma preparation used as a positive control in neutralization studies was a 3-M KCl extract from a surgically removed tumour mass from the pelvis of a girl (CEA content 40 ng per mg protein [21]. By means of the MEM method the lymphocyte reactivity to 100–200 μg of this material can be detected in 87% of the cancer patients [22]. Thus this preparation is well suited as a control in CEA neutralization experiments since the amount of 4 ng CEA per single dose of 100 μg protein does not suffice for provoking CEA-specific lymphocyte responses. (The necessary threshold dose of CEA amounts to approximately 50–100 ng; for information see Table 2).

Antibodies and antiserum

Two monoclonal antibodies, MAB 23 and MAB 73 (murine isotype IgG_1), were a kind gift from Dr. J.-P. Mach, Ludwig Institute for Cancer Research, Lausanne, Switzerland. These antibodies are CEA-specific but do not share epitope specificity [23, 24]. MAB 23 has an affinity constant of $1.4 \times 10^8\ M^{-1}$ [23]. They were purified by ammonium sulphate precipitation and DE-52 chromatography from mouse ascites. The stock solutions contained 1 mg protein/ml. Control myeloma IgG_1 P_3-X68-AG8 was also a kind gift from Dr. Mach and was prepared from ascites in the same manner (1 mg/ml). The third CEA-specific monoclonal antibody was obtained from Hybritech, Europe, Liège, Belgium (IgG_1; 1 mg semi-purified antibody/ml, affinity constant $10^9 M^{-1}$): anti-CEA 107-4: rabbit hyperimmune serum to CEA 5/I (comparable to CEA Standard Dresden). The serum was absorbed with packed erythrocytes of the blood groups O, A and B (vol./vol. = 1/1) and, additionally, by liver perchloric acid extract powder (40 mg protein/ml). In order to prevent non-specific interactions with the biological system, the serum was furthermore exhaustively absorbed with pooled human lymphocytes and guinea pig peritoneal cells (in separate absorption steps, 1 vol. cell sediment/1 vol. serum).

Lymphocyte preparation and antigen incubation

Blood was drawn by venipuncture from patients with histologically verified colorectal carcinoma before surgery or any other treatment (0.5 ml 10% EDTA solution per 10 ml blood). Mononuclear cells were isolated by density centrifugation with dextran/Visotrast (dextran, mol. wt 60,000; 100 ml 6% solution mixed with 20 ml Visotrast. Visotrast is sodium diacetylaminotriiodobencoic acid). The method is comparable to the Ficoll-Hypaque technique of lymphocyte isolation. The cells were washed twice in PBS and made up to contain 10^6 lymphocytes per ml Eagle's medium without any addition. If not described otherwise, 1 ml (10^6 cells) lymphocyte suspension was incubated with 20 μl CEA solution at various concentrations or 100 μl teratocarcinoma extract (100 μg protein) for 90 min at 24°C. Control incubations without antigen were done with the respective volumes of saline. For neutralization experiments lymphocyte suspensions were adjusted to 2×10^6 cells per ml (see below). After incubation the cells were spun down and the supernatants frozen at -20°C until tested in MEM (1–3 weeks).

Macrophage electrophoretic mobility technique

Outbred guinea pigs were injected i.p. with 20 ml sterile liquid paraffin. By 8–14 days thereafter the peritoneal cells were harvested by washing the peritoneal cavity with PBS. After one wash with PBS the cells were irradiated with 200 rad (2 Gy) from a ^{60}Co source (this is believed to be necessary if the cells from several animals are to be pooled). After pooling the cells were washed once again with PBS and resuspended in Eagle's medium at a concentration of 10^7 macrophages per 3 ml. Three millilitres of macrophage suspension were mixed with 1 ml lymphocyte supernatant and incubated for 90 min at 24°C. Macrophage blank controls were supplemented with 1 ml medium. The electrophoretic mobility of 15–20 macrophages per sample was measured using 'Parmoquant' cell electrophoresis apparata (Carl Zeiss, Jena, G.D.R.) with optical display, manual operation manner and on-line data processing. The percentage of slowing was calculated in comparison to the mean value of macrophages blank control.

Neutralization experiments

Five hundred microlitres of antigen solution (250 ng CEA 5/II or 500 μg teratocarcinoma protein) were mixed with 500 μl of the respective dilution of antibody, control IgG_1 or antiserum and allowed to stand for 4 hr at room temperature. Thereafter the mixtures were kept at 4°C overnight. Whereas the MABs 23 and 73 as well as the control IgG_1 could be used without further treatment, the Hybritech MAB had to be dialysed to remove azide.

The anti-CEA 107-4 was absorbed as described above. For controls, CEA and teratocarcinoma protein were mixed with saline and kept under identical conditions. Antibody and antiserum dilutions without antigen were also prepared in the same manner for control purposes to rule out non-specific effects on the indicator macrophages.

Aliquots of 200 μl of each neutralization mixture were added to 500 μl lymphocyte suspension (10^6 cells), corresponding to an input of 50 ng CEA or 100 μg teratocarcinoma protein respectively. Incubation was performed for 90 min at 24°C. After centrifugation 600 μl supernatant per sample were collected and incubated with 2.5 ml macrophage suspension in order to measure lymphokine activity by means of the MEM technique. The resulting volumes of 3.1 ml are just sufficient to fill the system of 'Parmoquant'. In preliminary experiments the neutralization step was followed by co-precipitation of immune complexes and unbound IgG (Protein-A-Sepharose coated with anti-mouse IgG). Since the results obtained with these probes did not differ substantially from those of samples with simple neutralization, all further experiments were done with neutralization only.

Fractionation of lymphocyte supernatants

Lymphocytes from two colorectal cancer patients were incubated with 200 ng CEA 11 per 10^6 cells in 1 ml as described above. The lymphocyte blank supernatants were reconstituted for CEA 11 after incubation. The lymphokine activity of the supernatants from each patient was checked before pooling. The pooled supernatants (85 ml CEA and 75 ml CEA reconstituted control) were lyophilized, reconstituted with water, dialysed against water and fractionated on a Sephadex G-100 column (K 16/100, Pharmacia, Uppsala, Sweden). Extinction was recorded at 280 and 206 nm. The column was calibrated with dextran blue (mol. wt 200,000), bovine serum albumin (mol. wt 67,000), ovalbumin (mol. wt 43,000), chymotrypsin (mol. wt 25,000) and cytochrome *C* (mol. wt 12,500). The PBS effluent was collected in four arbitrarily chosen fractions of mol. wt: >100,000–85,000 <85,000–47,000, <47,000–17,000 and <17,000–3000, since no elution profile could be recorded on the base of extinctions. After lyophilization the fractions were reconstituted with 1 ml water and dialysed against water for 24 hr at 4°C. The fractions of the two pooled supernatants were adjusted with water such that 25 μl of each fraction corresponded to 1 ml (10^6 lymphocytes) of the original supernatant pool. For testing the biological activity, 1-, 10-, 50- and 100-μl volumes of the fractions were added to 3 ml macrophage suspension and incubated for cell electrophoresis as described above.

RESULTS

Dose-dependent reactivity of lymphocytes to three different CEA preparations

As can be seen from Table 2, the lymphocytes from 5 patients with colorectal cancer responded to all three CEA preparations used. The threshold dose necessary to get reponses differed with the three preparations. Whereas reactivity to CEA 5/II started with 10 ng in one patient and reached

Table 2. Threshold amount CEA in correlation to the total amount of dry substance of different preparations inducing positive MEM results

CEA preparation code No.	ng CEA per μg dry substance per incubation sample		MEM-results: % slowing ($\bar{x}$ ± S.D.)	MEM-results: No. of responses >5% / No. of patients tested
IAC 5/II*	10	0.045	1.4 ± 1.5	1/5
Standard	50	0.225	6.4 ± 3.8	3/5
Dresden	100	0.450	8.0 ± 0.8	5/5
	200	0.900	8.4 ± 2.1	5/5
	400	1.800	7.8 ± 3.1	5/5
First British	10	2.126	2.2 ± 1.9	0/5
Standard for	50	10.630	10.0 ± 1.4	5/5
CEA†	100	21.260	7.8 ± 1.9	5/5
	200	42.520	9.6 ± 3.2	5/5
2-34*	10	25.000	1.6 ± 1.5	0/5
	50	125.000	2.6 ± 1.9	1/5
	100	250.000	6.0 ± 3.1	3/5
	200	500.000	7.8 ± 2.3	4/5
	400	1,000.000	10.2 ± 1.8	4/5

* CEA content determined by means of the standard assay [19].

† CEA content according to data from Laurence *et al.* [20]. The high amount of dry substance in this preparation is due to added lactose [20].

its full height in all 5 patients with 100 ng, the First British Standard for CEA showed a clear-cut threshold of full response in all the 5 patients with 50 ng.

CEA 2-34 gave increasing responses between 50 and 200 ng, but one patient's lymphocytes did not react even to 400 ng, though responding to the other CEAs. Considering the 3 CEAs, the approximate threshold to obtain lymphocyte responses amounts to 50–100 ng CEA. This is a narrow range, especially as, in terms of dry substance, the British Standard is 47 times that of IAC 5/II, and CEA 2-34 is even 555 times that of IAC 5/II (Table 2). Nevertheless, some qualitative differences of the CEAs may play a role, because one patient's lymphocytes did not react even with 400 ng CES 2-34, but responded to both IAC 5/II and the British Standard.

Neutralization experiments

As demonstrated graphically in Fig. 1, the activity of CEA could be neutralized by the three MABs as well as by anti-CEA antiserum 107-4. Though the titration curves resulting from the three MABs seem to differ in some details, they can be considered to be very similar in principle. The high standard deviations, especially at the slope of the curves (antibody 1:40,000), are due to individual differences of the patients' lymphocytes. The specificity of these results is underlined by the missing neutralizing capacity of the myeloma IgG_1 on the one hand and the failure of all the reagents used to neutralize the teratocarcinoma protein on the other. This argument will not be weakened by some non-specific interactions of CEA or teratocarcinoma protein with myeloma IgG_1, a phenomenon which can easily be discriminated from the specific neutralization curve pattern. The same holds for some non-specific effects found with teratocarcinoma protein and anti-CEA 107-4 or MAB Hybritech.

Lymphokine activity in Sephadex G-100 fractions from CEA-induced pooled supernatants

These experiments were designed to investigate the possibility of mediator enrichment within certain fractions. Information on the lymphokine detected with the current assay in the human tumour system is not sufficient yet. The results of repeated tests are listed in Table 3 and show

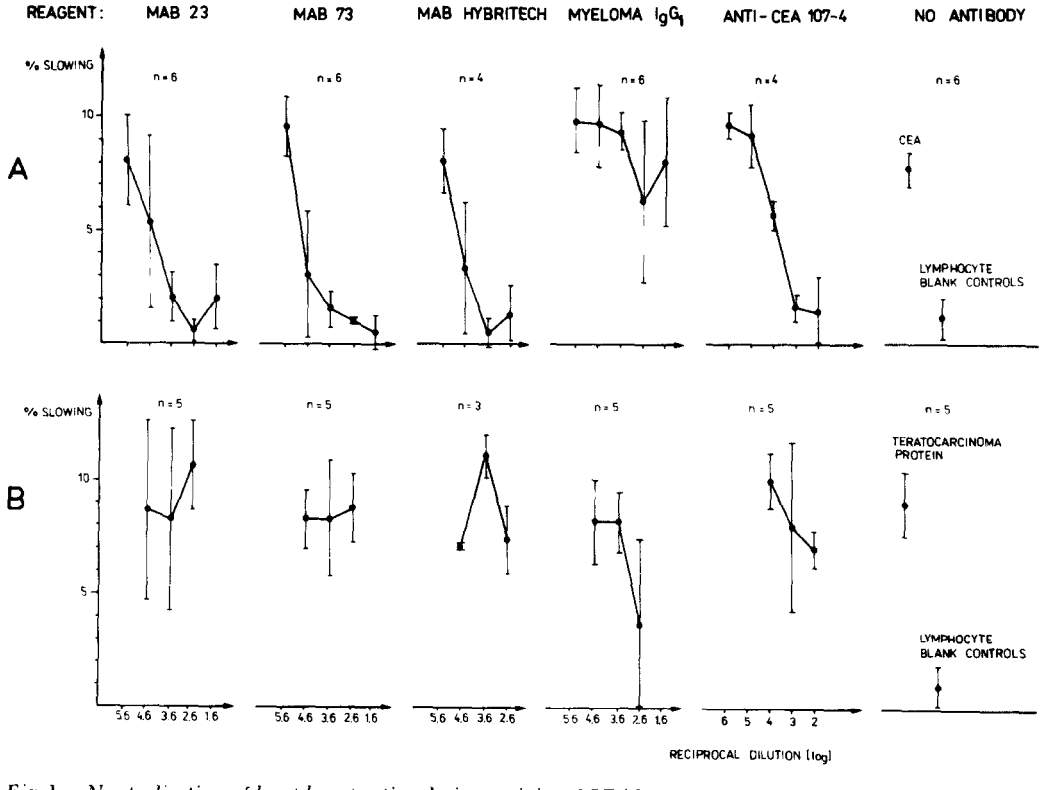

Fig. 1. Neutralization of lymphocyte-stimulating activity of CEA by monoclonal antibodies and an anti-CEA antiserum as revealed by the MEM technique. Antibodies and control myeloma IgG_1 were diluted 1:40–1:400,000. *Anti-CEA antiserum* 107-4 *was used at dilutions of* 1:100–1:1,000,000. *CEA and teratocarcinoma protein were allowed to react with these reagents before incubation with lymphocytes from colorectal cancer patients. The highest dilution steps of reagents were not tested with teratocarcinoma protein. A = experiments with CEA; B = experiments with teratocarcinoma protein. % slowing = lymphocyte supernatant-induced slowing of macrophages as compared to the mean value of macrophage blank controls.* n = *number of colorectal cancer patients (all tests have been performed with one group of 6 patients. Because of the limited lymphocyte yield, some titrations could be tested only in 5, 4 or 3 patients respectively).*

Table 3. Effects of Sephadex G-100 fractions of pooled lymphocyte supernatants on macrophage electrophoretic mobility

Material and volumes added to 3 ml cell suspension	% slowing of macrophage mobility [$\bar{x}$ ± S.D. (*n*)] CEA-induced lymphocyte supernatants	CEA-reconstituted lymphocyte control supernatants
Pooled supernatants, 1 ml	8.0 ± 1.4 (2)	1.7 ± 1.2 (3)
mol. wt fraction >85,000,* 1–50 μl	2.2 ± 1.6 (4)	2.3 ± 0.7 (4)
mol. wt fraction <85,000–47,000,* 1–50 μl	2.9 ± 2.2 (8)	1.6 ± 1.1 (8)
mol. wt fraction <47,000–17,000,† 1–100 μl	5.9 ± 2.7 (23)§	2.0 ± 1.7 (19)§
50–100 μl‡	4.3 ± 1.9 (7)	1.8 ± 1.4 (6)
10 μl	6.3 ± 2.6 (10)	1.7 ± 2.0 (9)
1 μl	7.2 ± 3.2 (6)	2.8 ± 1.4 (4)
mol. wt fraction <17,000–3,000,† 1–100 μl	4.9 ± 2.6 (15)‖	1.7 (12)‖
50–100 μl‡	3.7 ± 2.4 (5)	2.4 ± 1.7 (5)
10 μl	4.0 ± 1.7 (6)	0.5 ± 0.8 (5)
1 μl	7.7 ± 2.3 (4)	2.7 ± 2.8 (2)

n = No. of separate incubations tested.
*Both fractions >85,000 and <85,000–47,000 have not been tested in 100 μl amounts. Results with 1, 10, and 50 μl have been averaged.
†Results with 1, 10, 50 and 100 μl have been averaged.
‡Results with 50 and 100 μl have been averaged.
§Difference between identical fractions of both supernatants is significant with $P<0.001$ (Student's *t* test).
‖Difference between identical fractions of both supernatants is significant with $P < 0.02$.

clearly that the biological activity was recovered and enriched in two arbitrarily cut fractions of the mol. wt range 3000–47,000. No such activity was found in any fraction of lymphocyte control supernatant which had been reconstituted with CEA. Because of their low protein content the supernatants gave no elution profiles. Therefore the fractions were cut arbitrarily. Interestingly, there appeared to be an inverse dose-dependency since decreasing amounts of the active material elicit slightly increased macrophage slowing. But fractionation has not only resulted in slowing factor enrichment. There is an apparent gain of macrophage slowing activity since 1 μl active fraction corresponds to 40 μl of original supernatant, a volume of supernatant which does not induce mobility changes of macrophages.

DISCUSSION

From our studies on 499 individual patients and controls (Table 1) we have concluded that human lymphocytes can, in principle, respond to CEA. The main argument for this derives from the disease-associated response patterns which show that patients with cancer of the digestive tract frequently possess CEA-reactive lymphocytes. The aim of the experiments described in this paper was to investigate the crucial question of whether CEA itself or another substance co-purified with CEA may be responsible for the reactions observed. In the first series of experiments we compared the threshold amount of CEA necessary to induce lymphocyte responses using three preparations with different CEA contents. In all three preparations the threshold dose was found to be 50–100 ng CEA, though their CEA contents varied by factors of 47 and 555 respectively. Thus it seems most likely that the CEA itself is the active component. Individual lymphocyte samples showed somewhat different threshold sensitivities to the three CEA preparations and one patient's lymphocytes did not react at all to CEA 2-34, though responding to the two other preparations. This could mean that the well-known inhomogeneity of different CEAs [25–29] might also be reflected at the level of CEA-specific sensitization and response of human lymphocytes. The CEA-specificity of the lymphocyte responses described here has been definitely proven by the neutralization experiments. Three MABs with well-documented CEA-specificity abolished the activity of our standard CEA at high dilutions. The same effect was achieved by an

anti-CEA antiserum, which confirms our recent findings with other anti-CEA antisera [17]. Neither the MABs nor the anti-CEA serum substantially affected the activity of the teratocarcinoma extract, thus pointing to the specificity of the neutralization findings. Furthermore, the specificity of the data is underlined by the missing neutralization effect of the myeloma control IgG_1. The MABs 23 and 73 are known to differ with regard to their epitope specificity [23, 24]. Therefore the very similar neutralization capacity of both antibodies when tested with CEA in lymphocytes from 6 individual colorectal cancer patients is an unexpected finding. One explanation could be that a CEA-specific antibody, if bound to the molecule, prevents it from contact with lymphocyte receptors, possibly by steric interactions, regardless of the epitope specificity of the antibody and the lymphocyte receptors. Alternatively, the human recognition repertoire for CEA could differ from that of the mouse. In this sense one could hypothesize that two distinct epitopes of CEA to which the mouse produces different antibodies could be attributed to a region at the molecule, being recognized as single epitope by human lymphocytes. Whatever the right explanation will be, we can state, at least, that 3 MABs to CEA prevent CEA from inducing responses in human lymphocytes. This means that the active component of our standard CEA eliciting responses in human lymphocytes bears CEA-specificity.

The underlying mechanisms of the MEM technique employed for our studies is not well understood, especially the kind of lymphocyte response measured. Jenssen *et al.* [10] have shown that blood lymphocytes from PPD-sensitive guinea pigs release mediator(s) of an approximate mol. wt of 13,000 during the first 2 hr of incubation with PPD. These mediator(s) reduced the electrophoretic mobility of guinea pig macrophages. Kotzsch *et al.* [8] in our laboratory showed that Con A-induced lymphocyte supernatants from both human beings and guinea pigs caused guinea pig macrophage to slow in electophoresis. These findings clearly demonstrated that this kind of lymphokine action is not restricted to allogenic systems, as claimed by Petty *et al.* [30]. Continuing this work, Kotzsch *et al.* were able to enrich the charge-reducing guinea pig lympohokine(s) by Sephadex G-100 chromatography. Although the results of this latter large-scale study will be detailed elsewhere, it should be mentioned that the lymphokine(s) could be found in the 15,000 mol. wt region and was inhibited by α-L-fucose, whereas other monosaccharides were not inhibitory. As detailed in this paper, charge-reducing lymphokine activity of CEA-induced lymphocyte supernatants from colorectal cancer patients could be enriched within 2 out of 4 arbitrarily cut Sephadex G-100 fractions comprising the 3000–47,000 mol. wt range. Identical fractions from lymphocyte control supernatants reconstituted with CEA failed to show any activity. Thus these preliminary experiments clearly document that a biologically active mediator with a certain degree of stability will be released upon CEA contact *in vitro* by human lymphocytes. These lymphocytes seem to be obviously sensitized *in vivo*. Experiments conducted by our group with teratocarcinoma protein-induced lymphocyte supernatants from cancer patients, which will be published elsewhere, gave identical results with respect to the mol. wt region of lymphokine activity. The same phenomenon as has been described for the CEA system was found, namely that fractionation of supernatants resulted in a real gain of biological activity with an inverse dose–effect relationship. Possibly, fractionation leads to a separation of contrary activities from those with charge-reducing capability. Alternatively, some inactive or latent forms of the lymphokine(s) [31] could have been activated during certain steps of the whole separation procedure. Experiments have now been started to clarify this interesting phenomenon. To date, nothing is known on the lymphocyte subsets reactive to CEA. Therefore investigations are in preparation to characterize the lymphocyte populations involved in the reactivity to CEA.

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REFERENCES

1. LEJTENYI MC, FREEDMAN SC, GOLD P. Response of lymphocytes from patients with gastrointestinal cancer to the carcinoembryonic antigen of the human digestive system. *Cancer* 1971, **28**, 115–120.
2. VAN KLEIST S, BURTIN P. On the specificity of autoantibodies present in colon cancer patients. *Immunology* 1966, **10**, 507–515.

3. LoGerfo P, Herter FP, Bennett SJ. Absence of circulating antibodies to carcinoembryonic antigen in patients with gastrointestinal malignancies. *Int J Cancer* 1972, **9**, 344–348.
4. Gold P. Circulating antibodies against carcinoembryonic antigens of the human digestive system. *Cancer* 1967, **20**, 1663–1667.
5. Gold JM, Freedman SO, Gold P. Human anti-CEA antibodies detected by radioimmunoelectrophoresis. *Nature New Biol* 1972, **239**, 60–62.
6. Müller M, Irmscher J, Fischer R, Heidl G, Grossmann H. Immunological tumour profile, organ-specific carcinoma diagnosis in patients employing the macrophage electrophoretic mobility test. *Cancer Lett* 1977, **2**, 139–146.
7. Müller M, Irmscher J, Grossmann H *et al.* Antigen preparations with tumour site specificity. In: Preece AW, Sabolović D, eds. *Cell Electrophoresis: Clinical Application and Methodology*. Amsterdam, Elsevier, 1979, 219–224.
8. Kotzsch M, Grossmann H, Irmscher J, Müller M. Studies on lymphokine activity in concanavalin A-stimulated lymphocyte supernatants using the macrophage-electrophoretic-mobility-test (MEM-test). *Exp Pathol* 1982, **21**, 143–148.
9. Metzner G, Jäger L. Nachweis der Freisetzung des MSF durch Phythämagglutinin mit dem MEM-Test. *Acta Biol Med Ger* 1980, **39**, 223–227.
10. Jenssen HL, Werner H, Köhler H. Lymphokines which influence electrophoretic mobility of macrophages. *Acta Biol Med Ger* 1976, **35**, 1499–1503.
11. Kotzsch M, Irmscher J, Fischer R, Heidl G, Müller M. Untersuchungen zur immunologischen Spezifität des Makrophagen-Elektrophorese-Mobilitätstests bei Mäusen mit syngenen und allogenen Mammakarzinom-Transplantaten. *Acta Biol Med Ger* 1976, **35**, 1747–1753.
12. van Broen B, Albrecht S, Gryschek G *et al.* Cellular sensitization in man and mice during pregnancy to fetal antigens as detected by lymphokine assays. *Arch Geschwulstforsch* 1981, **51**, 371–374.
13. Pasternak G, van Broen B, Schlott B *et al.* Lymphocyte reactivity in animal and human tumour systems to fetal antigens as detected by MEM, LAI, and LMI techniques. In: Preece AW, Sabolović D, eds. *Cell Electrophoresis: Clinical Application and Methodology*. Amsterdam, Elsevier, 1979, 225–234.
14. Pasternak L, Jenssen HL, Köhler H, Pasternak G. Cross reactions among mouse tumours of different etiology as detected by macrophage electrophoretic mobility (MEM) test. *Eur J Cancer* 1976, **12**, 389–393.
15. Jenssen HL, Redmann K, Köhler H. The effect of a mediator of cellular immunity on the transmembrane potential of macrophages. *Acta Biol Med Ger* 1975, **34**, 1907–1910.
16. Valet G, Jenssen HL, Krafft M, Ruhenstroth-Bauer G. Flow-cytometric measurements of the transmembrane potential, the surface charge density and the phagocytic activity of guinea pig macrophages after incubation with lymphokines. *Blut* 1981, **42**, 379–382.
17. Müller M, Irmscher J, Heidl G, Grossmann H, Wagner H, Kotzsch M. Sensitization of human lymphocytes to the carcinomebryonic antigen (CEA): neutralization experiments with anti-CEA sera. *Exp Pathol* 1982, **21**, 5–9.
18. Grossmann H, Heidl G, Müller M. Zur Isolierung des carcinomebryonalen Antigens. *Acta Biol Med Ger* 1975, **34**, 1347–1357.
19. Zotter St, Heidl G, Grossmann H, Müller M, Johannsen BA, Pilz C. Radioimmunological characterization of carcinoembryonic antigen (CEA) preparations. *Exp Pathol* 1982, **21**, 10–20.
20. Laurence DJR, Turberville SG, Anderson SG, Neville AM. First British Standard for carcinoembryonic antigen (CEA). *Br J Cancer* 1975, **32**, 295–299.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
22. Irmscher J, Grossmann H, Kotzsch M, Wagner H, Müller M. Reactivity of human lymphocytes to teratoma preparations as revealed by the macrophage electrophoretic mobility (MEM) technique. *Arch Geschwulstforsch* 1982, **52**, 349–353.
23. Accolla RS, Carrel S, Mach JP. Monoclonal antibodies specific for carcinoembryonic antigen and produced by two hybrid cell lines. *Proc Natl Acad Sci USA* 1980, **77**, 563–566.
24. Buchegger F, Mettraux C, Accolla RS, Carrel S, Mach JP. Sandwich enzyme immunoassay using three monoclonal antibodies against different epitopes of CEA. *Immunol Lett* In press.
25. Banjo C, Shuster S, Gold P. Intermolecular heterogeneity of carcinoembryonic antigen. *Cancer Res* 1974, **34**, 2114–2121.

26. GOLD P, FREEDMAN SO, SHUSTER J. Carcinoembryonic antigen. Historical perspective, experimental data. In: HERBERMAN RB, MCINTYRE KR, eds. *Immunodiagnosis of Cancer*. New York, Marcel Dekker, 1979, 147–164.
27. PLOW EF, EDGINGTON TS. Specification of carcinoembryonic antigen and its immunodiagnostic implications. In: HERBERMAN RB, MCINTYRE KR, eds. *Immunodiagnosis of Cancer*. New York, Marcel Dekker, 1979, 181–239.
28. VRBA R, ALPERT E, ISSELBACHER KJ. Carcinoembryonic antigen: evidence for multiple antigenic determinants and isoantigens. *Proc Natl Acad Sci USA* 1975, **72**, 4602–4606.
29. VRBA R, ALPERT E, ISSELBACHER KJ. Immunological heterogeneity of serum carcinoembryonic antigen (CEA). *Immunochemistry* 1976, **13**, 87–89.
30. PETTY HR, WARE BR, RENOLD HG, ROCKLIN RE. The effects of stimulated lymphocyte supernatants on the electrophoretic mobility distribution of peritoneal macrophages. *J Immunol* 1980, **124**, 381–387.
31. POSTLETHWAITE AE, KANG AH. Latent lymphokines: isolation of guinea pig latent lymphocyte-derived chemotactic factor for monocytes. Its activation by trypsin and a soluble factor from macrophages. *J Immunol* 1979, **123**, 561–567.