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Molecular Species of Epidermal Growth Factor Carrying Immunosuppressive Activity

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The suppression of antibody formation to sheep red cells in mice by partially purified fractions of mouse submaxillary gland [7] was shown to be caused by epidermal growth factor (EGF). Purification of EGF by the method of Savage and Cohen [11] resolved three components referred to as EGF *a*, EGF *b*, and EGF *c*. All three induced premature eye opening in neonatal mice, but only EGF *a* (identified as EGF $_{1-53}$) had full immunosuppressive activity. EGF *c* was shown by micropeptide mapping of chymotryptic and thermolytic digests and amino-terminal analysis to differ from EGF *a* only by the presence of β-aspartyl instead of an asparaginyl residue. EGF *b* differed from EGF *a* in that it lacked the N-terminal asparagine. EGF shortened enzymatically at its carboxy terminal by two or five amino acids did not have any immunosuppressive activity. These findings suggest that, in contrast to some other biological effects of EGF, intact amino and carboxy terminals are required for the expression of immunosuppressive activity.

Key words: epidermal growth factor, heterogeneity, immunosuppressive activity

Several hormone-like substances that control growth and differentiation of various tissues are known to accumulate in the submaxillary gland of male mice. Apart from the well-characterized activities of nerve growth factor (NGF) and epidermal growth factor (EGF), extracts of these glands have also been shown to induce morphological changes in the thymus and peripheral lymphoid tissues [1–5] and suppression of some immune reactions [6–8]. A profound effect on the immune response to sheep erythrocytes (SRBC) by fractionated extracts of these glands has been reported from this laboratory [7]. Such fractions, given to mice optimally 24 hr before immunization with SRBC, reduced the number of specific antibody-producing spleen cells tenfold or more, abolished the switch from IgM to IgG antibody, but did not prevent the development of immunological memory. The secondary response, however, could also be abolished by treatment with the fractions 24 hr before boosting.

The fraction that possessed the bulk of the immunosuppressive activity contains proteins of approximately 70,000 M_r, with the high molecular weight EGF complex

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(HMW-EGF) as its major component. Preliminary experiments in which purified HMW-EGF was used showed that the activity is carried by this complex.

In the present communication we investigate the activity of EGF and EGF-derived peptides and report on the molecular form responsible for immunosuppression.

Some of this work was presented in preliminary form at the 12th International Congress of Biochemistry in Australia (August 1982).

MATERIALS AND METHODS

Animals

Random-bred Quackenbush mice (a line obtained originally by selection for fertility from Swiss Albino mice) were used in all experiments. Two-month-old males; (35–40g body weight) were used as a source of submaxillary glands and in immunological tests.

Bioassays

The bioassay for EGF in newborn mice, based on precocious tooth eruption and eyelid opening, was performed according to Cohen [9].

The immunization of mice with SRBC, the preparation of spleen suspensions, and details of the plaque-forming cell assay have been described previously [7].

Antibody against EGF was raised in New Zealand white rabbits and gel immunodiffusion performed according to Ouchterlony [10].

Preparation of EGF Peptides

EGF (also known as EGF $_{1-53}$) and EGF-2 (EGF $_{1-51}$, the peptide lacking the carboxy-terminal -Leu-Arg sequence of EGF) were prepared from acid extract of submaxillary glands by Bio-Gel P-10 (Bio-Rad, 100–200 mesh) and DE52 cellulose (Whatman) chromatography as described by Savage and Cohen [11]. EGF-5 (EGF $_{1-48}$ or T-EGF) was prepared from EGF $_{1-53}$ by tryptic digestion according to the method of Savage et al [12]. Reverse-phase, high-performance liquid chromatography (HPLC), similar to that described recently by Matrisian et al [13], was used to assist in the identification of the EGF peptides.

Preparation of EGF-Binding Protein

Submaxillary gland extracts were fractionated by chromatography on Sephadex G-75 and subsequent preparative polyacrylamide gel electrophoresis as described by Naughton et al [14]. The fraction containing electrophoretically pure EGF-binding protein was passed through a column of Sephadex G-50 equilibrated with 0.05 M Tris-HCl pH 8.1.

The binding protein prepared by this procedure had a specific activity of 378 μmol/min/mg at pH 9 and 25°C, with Nα-benzoyl-L-arginine ethyl ester (BAEE) as substrate.

Recombination of the binding protein with various EGF peptides and measurement of enzyme activity were performed according to Server et al [15].

Polyacrylamide Gel Electrophoresis

Discontinuous gel electrophoresis in glass tubes or slabs using 12% resolving gels and the bis-tris-TES system at pH 7.05 was carried out as described by Server

and Shooter [16]. The gels were stained with 0.5% Coomassie brilliant blue R250 in methanol-water (3:7) containing 5% trichloroacetic acid and 10% sulphosalycilic acid, and destained in 10% acetic acid.

Isoelectric focussing was performed according to O'Farrell [17] and sodium dodecyl sulfate (SDS) gel electrophoresis according to Laemmli [18].

Protein Estimations

Protein was determined by the method of Lowry et al [19]. EGF was also measured by the extinction coefficient of 30.9 ($E^{1\%}_{1cm}$ at 280 nm) [20].

Characterization of the EGF Peptides

As well as being characterized chromatographically and electrophoretically, the EGF peptides were analysed by micropeptide mapping, NH_2-terminal sequence determination, and amino acid compositional analysis [21].

Where necessary, each EGF peptide was oxidized with performic acid, then digested with α-chymotrypsin (Worthington) [12]. Chymotryptic peptides were separated by paper electrophoresis using either pH 2.6 buffer [12] or pH 2 buffer (formic acid:acetic acid:water, 2:8:90 by vol) under Varsol at 1,000 V for 1 hr. Cellulose thin-layer two-dimensional micropeptide mapping of the chymotryptic digests were undertaken as described by Whittaker and Moss [21].

Diagonal electrophoretic peptide maps to identify disulphide-linked peptides were carried out by a modification of the Brown and Hartley technique [22]. The EGF peptides were first digested at pH 6.5 with thermolysin as described by Savage et al [23]. The resulting peptide mixtures were separated on thin-layer cellulose plates by electrophoresis at pH 3.5 for 35 min at 1,000 V. After drying, the plates were exposed to an atmosphere of freshly made performic acid vapours for 2 hr [22]. The plates were aerated thoroughly and dried extensively in vacuo before electrophoresis again, at right angles to the first dimension. In other experiments, the thermolysin digests were oxidized prior to two-dimensional micropeptide mapping as for the chymotryptic digests.

The dried peptide maps were stained either with fluorescamine or with ninhydrin.

NH_2-terminal sequences and amino acid compositions of EGF variant peptides were determined as described previously [21].

RESULTS

Separation of EGF Peptides

A typical elution profile obtained at the final purification step on diethylaminoethyl (DEAE)-cellulose is shown in Figure 1. This pattern, similar to published data [11], was completely reproducible with aliquots of the same gland preparation, but showed some variability between preparations and more so with different batches of DEAE cellulose. The bulk of the peptide material was always in the fraction designated EGF *a*, yielding 20 to 25 mg/150 mice, while the quantity of EGF *c* was more variable, giving 4–8 mg. The greatest variation was in the elution volume and amount of EGF *b*. In some instances it was virtually absent or appeared as a small shoulder on the descending limb of EGF *a*.

Analytical polyacrylamide gels discriminated the three peptides. The relative mobilities of EGF *a* and EGF *b* were 0.52 and 0.49, respectively, while the more

acidic EGF *c* had an R_m of 0.62 (Fig. 1). Gels loaded with 20 μg or more of the EGF fractions occasionally revealed additional electrophoretic bands; EGF *a* as well as EGF *b* showed a faint component with an R_m identical to that of EGF-2, while EGF *a* showed a band of varying intensity corresponding to the mobility of EGF *c*. EGF *c* itself appeared as a single band.

EGF *a*, *b*, and *c* all migrated as a single molecular species of M_r about 6,000 during polyacrylamide gel electrophoresis in SDS under reducing conditions (5% 2-mercaptoethanol).

On isoelectric focussing EGF *a* gave a pI of 4.6 in agreement with the published values [20,24]. EGF *b* could not be distinguished from EGF *a*, while the more acidic EGF *c* had a pI of 3.9. Immunodiffusion analyses showed lines of antigenic identity for the three peptides.

Biological Activities of EGF Peptides

Since EGF *b* could be obtained only in small amounts, in the following experiments freshly prepared EGF *a* and EGF *c* were mostly used.

Table I shows that the EGF peptides are indistinguishable by the classical tooth-eruption and eyelid-opening assay of Cohen [9]. The assay for immunosuppressive activity, however, clearly discriminates EGF *a* and EGF *c*.

In the initial experiments the major component, EGF *a* was used. Immunosuppression was found to be maximal when the peptide was injected into mice 24 hr prior to immunization with SRBC, but absent or negligible when administered simultaneously with the antigen. A single injection of EGF *a* (400 μg/mouse) given 24 hr before immunization, caused on the average a ten-fold reduction in the number of

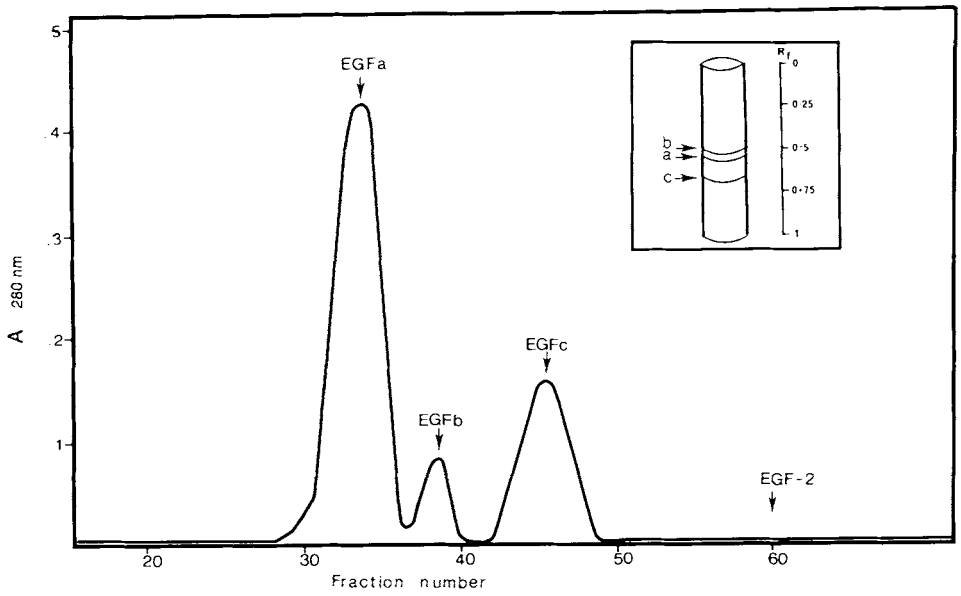

Fig. 1. Separation of EGF peptides by DEAE-cellulose chromatography. Prior to equilibration the DEAE-cellulose was placed into acetic acid (pH 4) and degassed. Insert shows a schematic representation of their separation in 12% polyacrylamide disc gels at pH 7.05.

TABLE I. Tooth-Eruption and Eyelid-Opening Assay*

Group	No. of animals	Age (in days)	
		Incisor eruption	Eyelid opening
Experiment 1			
Control	8	9–10	12–14
EGF *a*	8	7–8	8–9
EGF *b*	8	7–8	8–9
Experiment 2			
Control	8	9–10	12–13
EGF *a*	8	7–8	8–9
EGF *c*	8	7–8	8–9

*In each of the two experiments 24 newborn mice were distributed equally to two mothers, and each litter was divided into three groups. All animals were injected first at the day of their birth then every 24 hr for another 7 days. Freshly prepared EGF peptides were first concentrated by pressure ultrafiltration and then lyophilized. The material was taken up in a minimal volume of distilled water and diluted prior to use with physiological saline to contain 60 μg/ml of the peptide. Experimental animals received daily 1 μg/g body weight of the peptides; control animals received an appropriate volume of saline solution.

specific IgM-secreting cells of the spleen (see Table II), and an even stronger reduction in the number of IgG producers when measured at the peak of the primary IgG response (575 ± $71/10^6$ spleen cells in controls, versus 11 ± $8/10^6$ spleen cells in EGF *a*-treated animals).

Despite the strongly suppressed primary response, EGF *a*-treated animals gave a normal secondary response when boosted 6 wk later with SRBC, thus showing unimpaired immunological memory. The secondary response of these animals as well as that of normal controls could be abrogated by injection of EGF *a* (400 μg/mouse) 24 hr before boosting. These results demonstrate that the pattern of immunosuppressive activity of EGF *a* is identical to that described earlier [7] for high molecular weight fractions of submaxillary gland extracts.

When extended to a comparison of the activities of EGF *a* and EGF *c*, it was shown conclusively that EGF *a* was the major immunosuppressive fraction (Table II). Injection of 270 μg/mouse resulted in an approximate 80% suppression of the primary immune response and 400 μg caused the maximal suppression obtainable. In contrast, 400 μg of EGF *c*/mouse did not result in any significant suppression, and the highest dose of 1.2 mg (in 1 ml of saline) was not quite as effective as the lower dose of EGF *a*.

The activity of EGF *a* was also compared with EGF peptides lacking two or more carboxy-terminal amino acids (Table II, experiment 3). The results clearly indicate that an unaltered carboxyl terminus is necessary for immunosuppressive activity.

Biochemical and Chemical Studies on EGF Peptides

The high molecular weight form of EGF as found in the submaxillary gland can be reconstructed by recombining purified EGF with EGF-binding protein [25], thereby diminishing the esteropeptidase activity of the latter [15]. An intact carboxyl terminus of EGF is apparently essential for this reconstitution, since EGF derivatives lacking

TABLE II. Effect of EGF Peptides on the Primary Immune Response†

Experiment	Group	IgM plaques/10^6 lymphoid cells (Mean ± SE)
1	Control	1,085 ± 222
	EGF *a*, 270 μg	223 ± 96*
	EGF *a*, 400 μg	69 ± 15**
2	Control	986 ± 179
	EGF *c*, 400 μg	788 ± 100 NS[a]
	EGF *c*, 800 μg	444 ± 76*
	EGF *c*, 1,200 μg	272 ± 68*
3	Control	792 ± 42
	EGF *a*, 400 μg	75 ± 14**
	EGF-2, 400 μg	680 ± 163 NS
	EGF-5, 400 μg	813 ± 98 NS

†Groups of eight mice were injected i.p. with freshly prepared EGF peptides, which had been concentrated by ultrafiltration and passed through a Sephadex G-10 column equilibrated with 0.85% NaCl. Twenty-four hours later all animals received 2×10^8 SRBC i.p. They were sacrificed 4 days after immunization, and the IgM plaque-forming cells of their spleens were enumerated. Results are presented as arithmetic mean ± standard error for each group. Means were compared pairwise by Student's t-test (double tailed).

*$P \leqslant .01$.

**$P \leqslant .001$.

[a]Not significant.

either the terminal -Arg residue or -Leu-Arg sequence do not recombine with the binding protein.

In the present recombination experiments, following the method of Server et al [15], EGF *a*, EGF *b*, EGF *c*, EGF-2, and EGF-5 were used. The peptides were incubated in a 1:1 ratio by weight with the binding protein. Reconstitution was assessed by measuring the inhibition of esteropeptidase activity and also by visualization on polyacrylamide electropherograms.

Free EGF-binding protein hydrolysed BAEE at pH 7 at the rate of 180 μmol/min/mg. Neither EGF-2, as already shown by Server et al [15], nor EGF-5 inhibited the enzymic activity, thus indicating an inability to recombine with the binding protein through their altered carboxyl termini. It is of interest that EGF-5, which like EGF has -Leu-Arg at its carboxy terminal, did not recombine with the binding protein. This might indicate that either some of the other three amino acids are also required for binding or that the conformational changes [26] accompanying the removal of the carboxy terminal pentapeptide inhibit the recombination.

In contrast, the results of experiments with the EGF *a*, *b*, and *c* peptides showed that all three were equally able to recombine with the protein; in a number of experiments these peptides inhibited enzymic activity by 47–53%. Such results implicate similar unaltered carboxyl terminals in these three peptides.

Comparative Studies of the EGF Peptides

As seen in Figure 1, EGF *a* is chromatographically well separated from EGF *c*. However, electropherograms of EGF *a* always show a trace of a band corresponding to the mobility of EGF *c*. The proportion of the latter was found to increase on

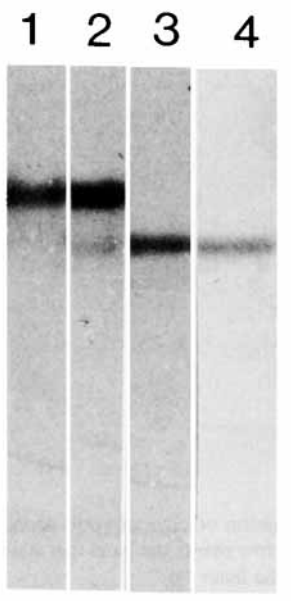

Fig. 2. Effect of mild alkali treatment on EGF. EGF *a* was incubated at pH 10 and 37°C. Polyacrylamide gel electrophoresis in 12% gels at pH 7.05 of aliquots 1) 0-hr, 2) 24-hr, 3) 48-hr incubation; and 4) EGF *c*.

prolonged standing. When fresh EGF preparations were deliberately incubated under alkaline conditions, progressive changes in concentration to the more acidic component occurred with time as shown electrophoretically (Fig. 2), and by DEAE cellulose chromatography (not shown). In addition, simply boiling EGF *a* for 15 min in distilled water, adjusted to pH 8, resulted in its partial conversion to the acidic form, migrating in the position of EGF *c*, while similar treatment left EGF *c* unchanged. This suggested that alkali-mediated deamidation was probably occurring. In order to substantiate this interpretation, comparative peptide mapping of proteolytic digests, amino acid composition and sequence analyses were used to locate and identify altered peptides.

The amino acid compositions of EGF *a* and EGF *c* were indistinguishable (data not shown) and agreed with that of the EGF purified by Taylor et al [20]. Dansyl-Edman microsequence analyses and automatic sequencing of intact EGF *a* and EGF *c* implicated a difference at the amino terminal since a sequence Asx-Ser-Tyr-Pro could only be obtained with the former. This sequence is consistent with the published data for EGF [12]. EGF *b* had the N-terminal sequence Ser-Tyr-Pro, suggesting that the N-terminal asparagine of EGF $_{1-53}$ was missing. Up to 20% of this peptide was also found as a contaminant in some preparations of EGF *a*. In these cases it was enriched on the descending limb of the EGF *a* chromatographic peak. EGF *c*, although containing amino terminal Asx, could not be sequenced at all.

For the comparison of chymotryptic peptides T-EGF was prepared from both EGF *a* and EGF *c* in order to avoid artifacts caused by the effect of performic acid on tryptophan. Figure 3 shows the paper electrophoretic separation of these peptides.

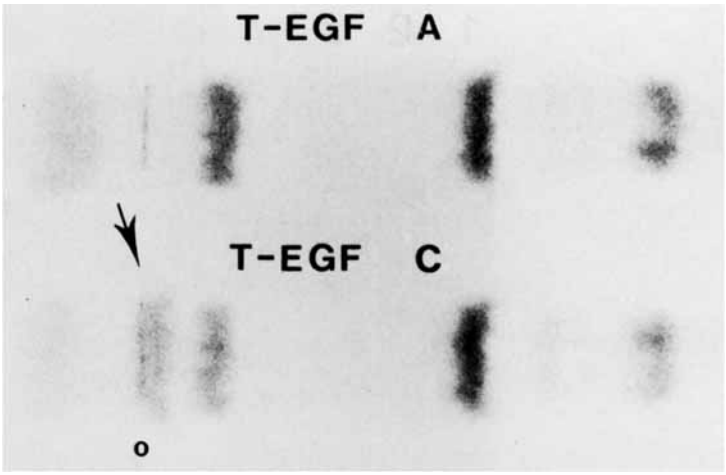

Fig. 3. Paper electrophoretic separation of chymotryptic peptides obtained from performic acid oxidised T-EGF *a* and T-EGF *c*. The arrow marks the band that stains blue with ninhydrin. The position of the application band is indicated by the letter "o."

The pattern obtained for T-EGF *a* agreed with that reported [12]. T-EGF *c*, however, showed at least one peptide variation. Since ninhydrin stained the variant zone a distinctive blue colour, a peptide with amino terminal β-aspartic acid was indicated [27]. The unmodified form of this peptide in T-EGF *a* comigrates with others in the first cathodic peptide zone (see strongly stained band).

In order to characterize the change(s) more fully, we undertook two-dimensional micropeptide mapping of performic acid-oxidized EGF *a* and EGF *c* rather than their T-EGF derivatives. Maps of both chymotryptic digests and thermolytic digests are compared in Figure 4. Thermolysin treatment generates, on average, tripeptides of the EGF so that altered peptides are readily detectable, especially in mixed maps of the components being compared. Figure 4 demonstrates the close relationship of EGF *a* and EGF *c* and reveals only one peptide difference between them. Additionally, this difference occurs in one of the expected three disulphide-linked peptides of EGF (Fig. 5), which would include the amino terminal sequence. The disulfide-linked peptide of EGF *a* that on performic acid oxidation generates peptide 3 and its partner (Fig. 5A) is altered in mobility in EGF *c* and gives rise to peptide 3c, which comigrates with its partner (Fig. 5B).

Fig. 4. Micropeptide maps of proteolytic digests of EGF *a* and EGF *c*. A) Chymotryptic digest of EGF *a*. B) Chymotryptic digest of EGF *c*, stained with fluorescamine. The variant peptide is marked with arrows. Peptide 1 in EGF *a* is missing in EGF *c*; instead, a peptide marked "1c" appears in EGF *c*, which stained blue with ninhydrin. C) Thermolytic digest of EGF *a*. D) Thermolytic digest of EGF *a* mixed with EGF *c*. E) Thermolytic digest of EGF *c*, visualized with ninhydrin. "2" marks the peptide in EGF *a* (map C), which is missing in EGF *c*, and "2c" marks the blue-staining peptide found in EGF *c* (map E).

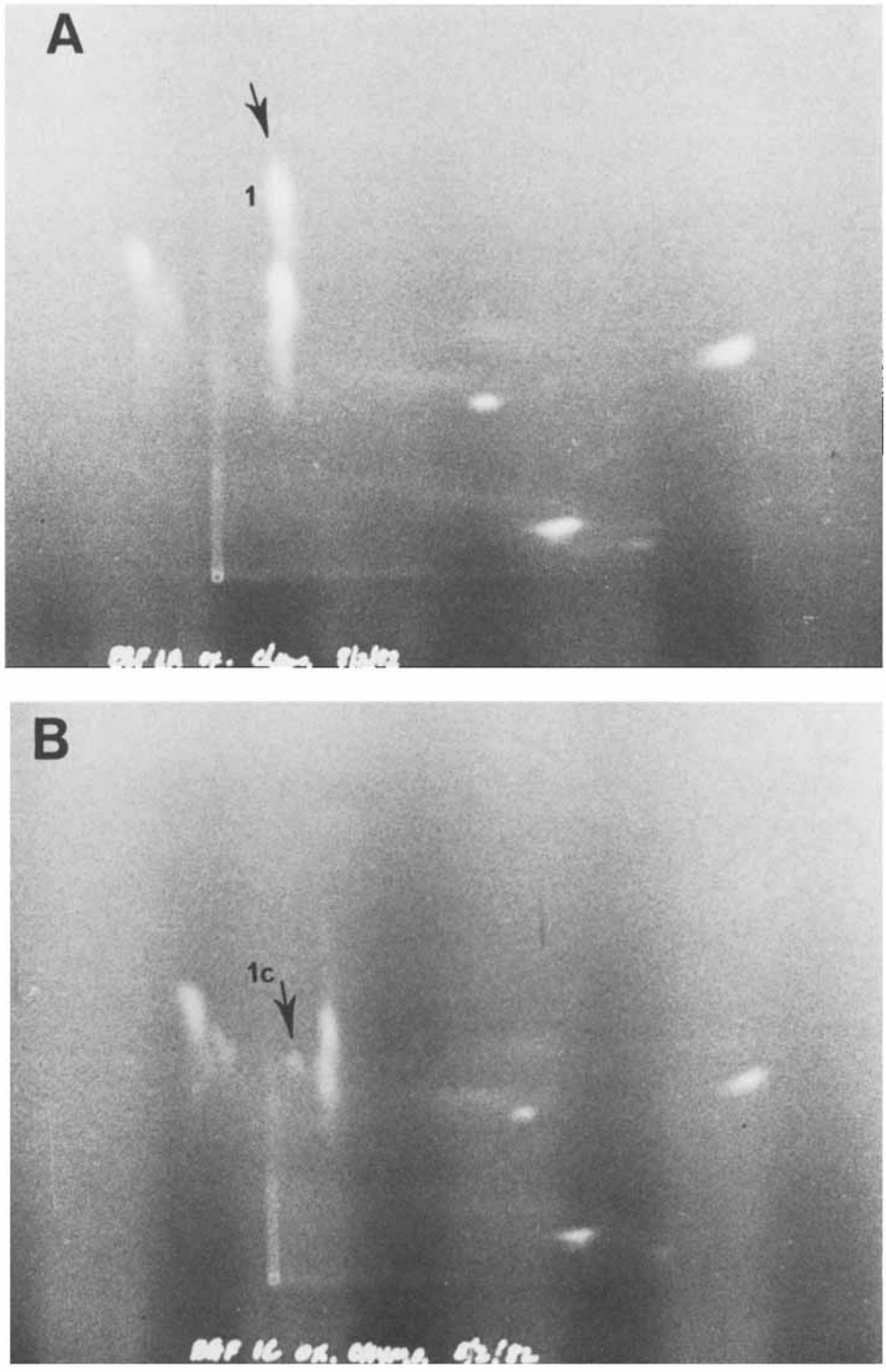

Fig. 4.

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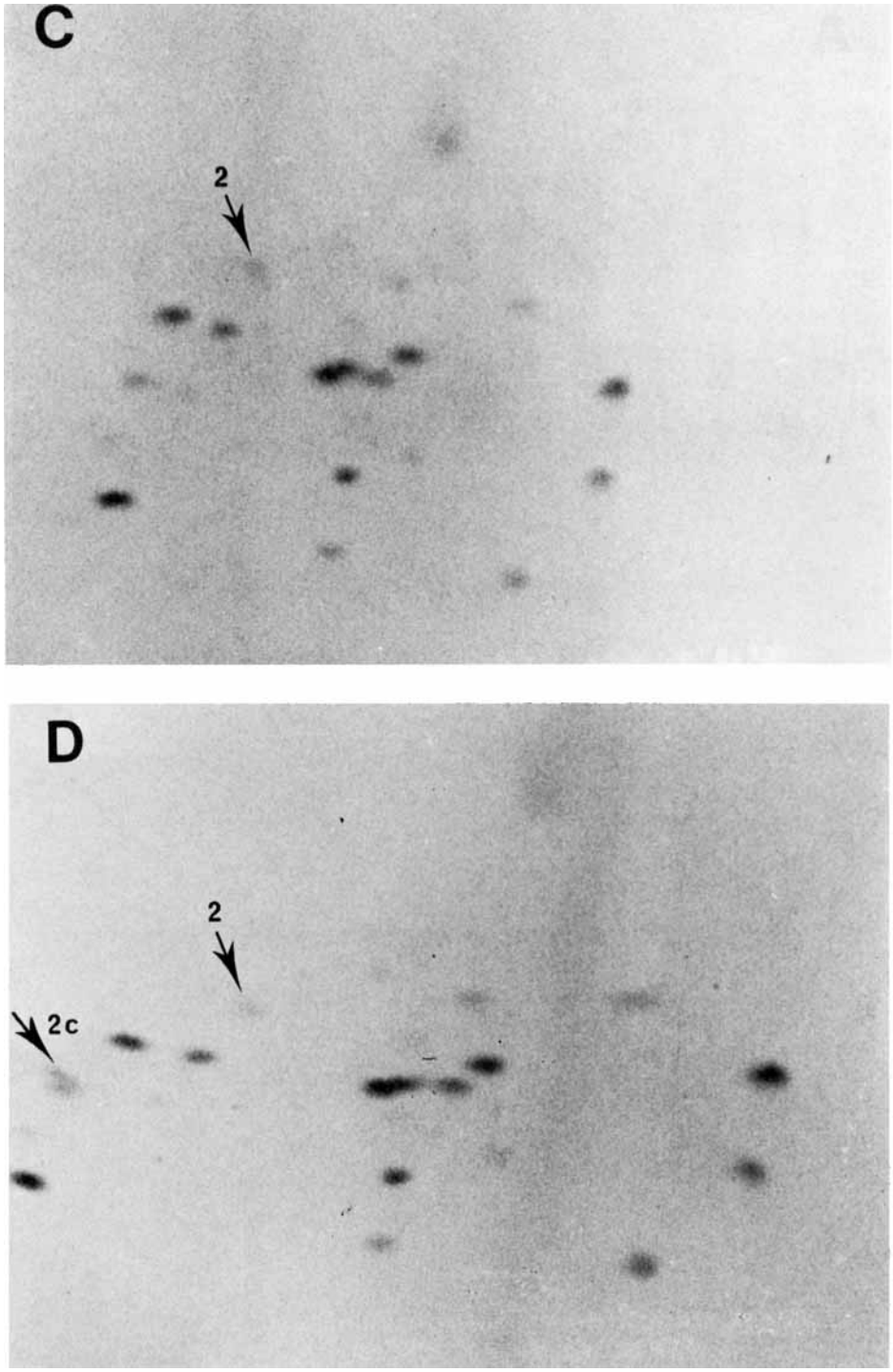

Fig. 4. (continued)

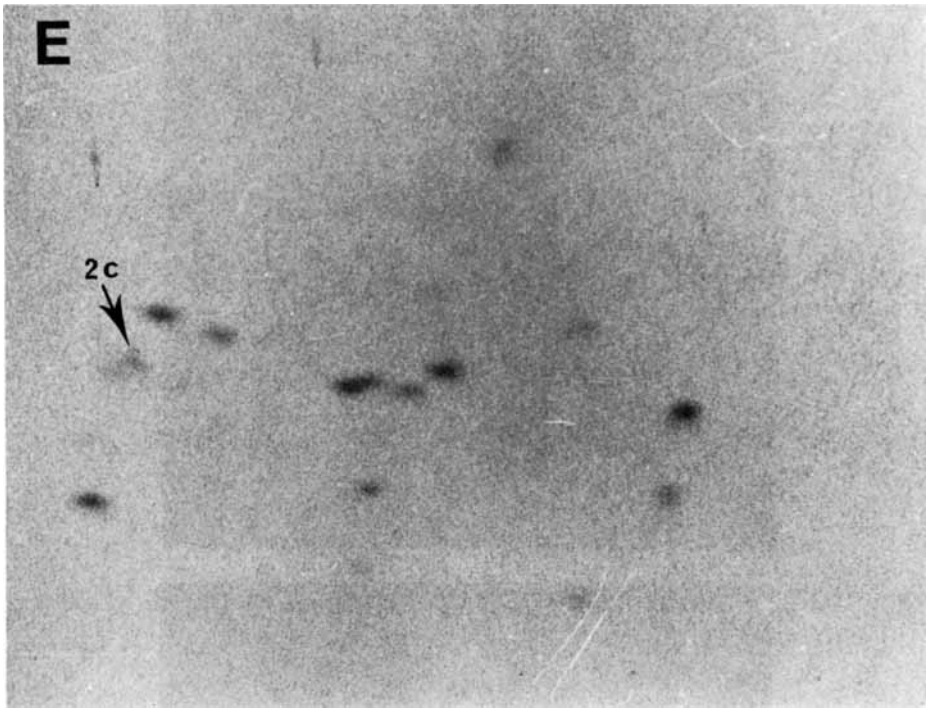

Fig. 4. (continued)

The acidic component, which was deliberately generated from EGF *a* at pH 10, and which migrated chromatographically and electrophoretically in the position of EGF *c*, was identical to EGF *c* by comparative peptide mapping of proteolytic digests.

In the work reported here, we found it necessary to prepare sufficient maps for staining with fluorescamine and ninhydrin, since the former reagent only weakly stained some peptides, including the variant chymotryptic and thermolytic peptide of EGF *c* (Fig. 4B,D), and failed to visualize others. Ninhydrin, as well as revealing the maximum number of peptides, stained the EGF *c* variant peptide with the characteristic blue colour of an amino-terminal β-aspartyl peptide. Ninhydrin also revealed the virtual comigration of the variant EGF *c* thermolytic peptide with another peptide (compare Fig. 4C,D) that interfered with subsequent analyses. We therefore analysed the well-resolved variant chymotryptic peptides.

The amino acid compositions of these peptides were indistinguishable, and were consistent with the amino terminal sequence Asn-Ser-Tyr-Pro-Gly-Cys-Pro-Ser-Ser-Tyr- of EGF [12]. Partial dansyl-Edman microsequencing of these peptides from EGF *a* and EGF *c* showed Asx-Ser-Tyr- in the former but only Asx- in the latter, which corroborated our results for the corresponding intact EGF molecules. Our inability to sequence the EGF *c* peptide is presumably due to deamidation of the asparagine with β-aspartyl peptide formation, which blocks cleavage at the phenylthiocarbamoyl step of the Edman degradation [28].

EGF-2 and EGF-5 (prepared from EGF *a*) differed from EGF *a* in chymotryptic peptide maps only in the basic carboxy-terminal peptides (not shown) as expected from the carboxy-terminal shortening of these EGF molecules.

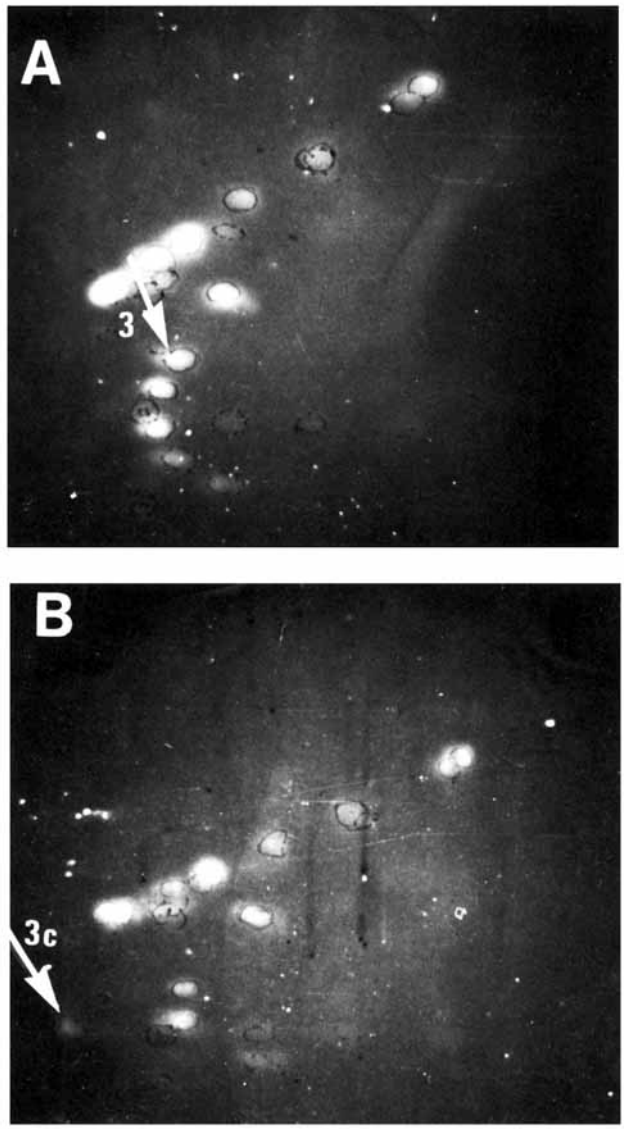

Fig. 5. Diagonal electrophoreses of thermolytic peptides of EGF *a* and EGF *c* visualised with fluroescamine. Peptide 3 disappears from EGF *a* (A), giving rise to the peptide 3c in EGF *c* (B), which stains blue when overstained with ninhydrin.

According to HPLC analysis using the conditions of Matrisian et al [13], EGF *a* preparations containing the contaminating EGF *b* peptide were resolved into two components. These correspond to the α-EGF and β-EGF of these authors. EGF *b* gave a single-peak equivalent to β-EGF, and EGF *c* also eluted as a single peak, which appears as a leading shoulder when cochromatographed with EGF *a*.

DISCUSSION

We have reported earlier the profound suppression of antibody production by partially purified extracts of mouse submaxillary glands [7]. The activity resides mainly in a 70,000 M_r fraction. We have now extended these findings by showing that purified small molecular weight EGF is responsible for the observed immunosuppression and by examining the activity of a number of EGF-derived peptides. Our results show that in contrast to other biological effects only the unaltered EGF molecule, EGF $_{1-53}$, carries full immunosuppressive activity.

Purification of EGF by gel filtration and ion exchange chromatography revealed in our hands a heterogeneity of this peptide. Besides the main component, EGF $_{1-53}$ (designated EGF *a*), we obtained in all preparations a more acidic peptide, EGF *c*, and a minor component, EGF *b*, which eluted after the main peptide and was variable in its resolution and quantity.

All three peptides behaved identically, as judged by their effect on eyelid opening and tooth eruption in newborn mice. There was no observable difference in their molecular weights and they were shown to be antigenically identical. Furthermore, all three EGF variants had unaltered carboxy termini, as shown by their ability to recombine with—and inhibit—the enzymic activity of EGF-binding protein. It is of interest, therefore, that only the main component EGF *a* carried full immunosuppressive activity. EGF *c*, in comparison, had only a weak effect, and in pilot experiments (not shown) EGF *b* had similar negligible activity.

Comparative micropeptide mapping of chymotryptic and thermolytic digests showed that the only difference between the highly active EGF *a* and the weak immunosuppressor EGF *c* resides in the deamidation of the amino terminal asparagine of the latter. The typical blue ninhydrin colour of the variant spot, found in both digests of EGF *c*, identified it as a β-aspartyl peptide bond and was substantiated by the blocking of the Edman degradation as noticed earlier [12].

Such changes at asparaginyl peptide bonds are known to occur easily and have been demonstrated also here by our intentional deamidation studies. Our experiments do not exclude, however, the possibility of this transformation occurring also in vivo. Deamidations in vivo have been reported [29,30], and it has been suggested that they might act as a means of altering the activity in the "life-cycle" of biologically active molecules [30]. The anomalous behaviour of EGF *b*, which appears to be more acidic than EGF *a* on ion exchange chromatography, but neither on analytical acrylamide gel electrophoresis nor on isoelectric focussing, is not yet understood.

Heterogeneity of EGF preparations has been reported earlier. Savage and Cohen [11] observed on ion exchange chromatography a minor peak, eluting as our EGF *c*, which they did not fully investigate. They mentioned, however, that it had similar biological activity and amino acid composition to EGF and was precipitable by anti-EGF antibody. This group [12] also obtained a contaminating amino terminal sequence Ser-Tyr-Pro in their EGF $_{1-53}$ preparations.

Petrides et al [31], purifying EGF by reverse-phase HPLC, reported the separation of two major components with EGF immunoreactivity but differing in their chymotryptic peptide fragments. Recently Burgess et al [24], also isolating EGF by HPLC, with the application of ion-pairing solvent, obtained a variant of slightly different amino acid composition that showed antigenic identity and similar biological

activity to EGF on newborn mice, but was considerably less potent in achieving maximal stimulation of DNA synthesis in 3T3 cells. Both authors showed their variants to be indistinguishable from EGF not only on SDS-polyacrylamide gels, but also on isoelectric focussing. Matrisian et al [13], using another HPLC fractionation method, also obtained two major EGF components, designated α-EGF and β-EGF. These authors found minor differences in the amino acid compositions, and electrophoretic mobilities in SDS-urea-polyacrylamide gels of the two peptides. Although both appeared to behave similarly in the newborn mice and EGF-receptor assays, they had different potencies in stimulating DNA synthesis in cultured cells, but were both more active than DEAE purified EGF in the latter assay system.

While changes of the amino terminus of EGF diminish its immunosuppressive activity, alteration at its carboxy end have an even more profound effect. Shortening of the carboxy terminus by either two or five amino acids abolishes the immunosuppressive activity. These shortened EGF peptides have been reported to retain the ability to cause precocious eyelid opening and tooth eruption [12] and EGF-receptor phosphorylation [32]. Linsley and Fox [33], however, found that while N-terminally modified biotinyl-EGF bound to 3T3 cells to the same extent as unmodified EGF, biotinyl-EGF shortened by five amino acids showed much diminished binding.

It can be concluded from the cited literature and from the present work that some changes in the EGF molecule leave certain of its biological effects intact, but affect others. Whether these differences of action reflect a nonuniformity of the receptors of the various target cells or are caused rather by differences in postinternalizational events is as yet not known.

The action mechanism of EGF on the immunoreactive cell(s) resulting in suppression of antibody production is presently being investigated.

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