Characterisation of the influence of anti-gastrin, anti-epidermal growth factor, anti-oestradiol, and anti-luteinising hormone releasing hormone antibodies on the proliferation of 27 cell lines from the gastrointestinal tract

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Abstract

Numerous data from published reports prove that the proliferation of gastrointestinal tumour cell lines are under the control of many hormones or growth factors, or both. Most of these publications report the influence on a very small number of cell lines of one or two such factors only. This work deals with the *in vitro* characterisation of the influence of the anti-gastrin, the anti-epidermal growth factor (EGF), the anti-oestradiol (E_2), and the anti-luteinising hormone releasing hormone (LHRH) antibodies on the proliferation of a large series of gastrointestinal cell lines. Cell proliferation was assessed by means of the colorimetric MTT assay on a series of 27 gastrointestinal cell lines obtained from the American Type Culture Collection (ATCC). Of the 27 cell lines, the anti-gastrin, the anti-EGF, the anti- E_2 , and the anti-LHRH neutralising antibodies considerably influenced the proliferation of 13, 25, 12, and 16. No gastrointestinal cell line was unresponsive to the four antibodies simultaneously. The anti-gastrin and anti-EGF antibody induced effects on the 27 gastrointestinal cell line proliferation were significantly correlated, as was also the case for the anti- E_2 and anti-LHRH antibody induced effects. Of the anti-gastrin, the anti-EGF, the anti- E_2 , and the anti-LHRH antibodies, it was the anti-EGF one that had the greatest influence, both quantitatively and qualitatively, on gastrointestinal cell proliferation. The correlation of the effects of definite anti-hormone antibodies is suggestive of a common mechanism of action for the corresponding hormones and casts some doubt on the efficiency of anti-hormone monotherapy. (Gut 1995; 36: 220-230)

Keywords: gastrointestinal tract, hormones, growth factors, cell lines.

It has become clearly established that gastrin plays an important part in the growth control of normal and malignant gastrointestinal mucosa¹⁻⁴ since the description of the presence

of gastrin receptors in rats' stomachs.⁵ This role is mediated through the gastrin receptors⁶⁻⁹ and is exerted by an autocrine action mechanism.¹⁰⁻¹² Furthermore, recent data show that gastrointestinal tumour cells, and especially colorectal ones, are also sensitive to hormones or growth factors, or both like the transforming growth factors alpha and beta,¹³⁻¹⁶ the insulin like growth factors I and II,^{13 17} the basic fibroblast growth factor,¹⁸ and many others. In addition to these growth factors two additional ones are also well known as stimulants of gastrointestinal cell line proliferation. These are oestradiol (E_2)¹⁹⁻²¹ and the epidermal growth factor (EGF),^{22 23} for both of which specific receptors exist in normal and neoplastic gastrointestinal mucosa.^{14 24-26} We recently showed that the luteinising hormone releasing hormone (LHRH) can also considerably modulate colorectal cell proliferation.²¹

Referring to the results contained in published works, Frucht *et al*²⁷ state that they are limited because it often happens that only a small number of tumours are studied, only one or two receptors are sought, and the effect on cell function is not investigated. In their study of 10 recently characterised human colon cancer cell lines undertaken to discover if they possessed receptors for any of 12 different gastrointestinal hormones or neurotransmitters, these authors²⁷ show on these cell lines the presence of several different receptors that are functional because occupation by selective agonists has changed the intracellular mediators. While they²⁷ consider it necessary to extend their studies to evaluate growth effects, we have taken this as the goal of this work, which deals with the *in vitro* characterisation of the influence of the gastrin, the EGF, the E_2 , and the LHRH on the proliferation of 27 gastrointestinal cell lines obtained from the American Type Culture Collection (ATCC). We preferred to add the anti-hormone or anti-growth factor antibody, or both in the culture medium rather than the hormone or growth factor itself because more information is available in this way. Indeed, a significant hormone or growth factor induced effect, or both on a given cell line proliferation shows that the proliferation of this cell line is 'sensitive' to the addition of this hormone or growth factor, but does not show if such proliferation was already under the influence of this hormone or growth

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TABLE I Description and origin of the 27 cell lines under study

Name	ATCC code	Species	Histopathology	Tissue of origin	Reference no
SW403	CCL230	H	C	Colon	31
AGS	CRL1739	H	C	Stomach	32
SW1463	CCL234	H	C	Colon	31
CH1ES	CCL73	G	N	Oesophagus	33
SW48	CCL231	H	C	Colon	31
DLD-1	CCL221	H	C	Colon	34
GPC-16	CCL242	GP	C	Colon	35
Colo320	CCL220	H	C	Colon	36
LS123	CCL255	H	A	Colon	37
SNU-C2B	CCL250	H	A	Caecum	38
HT-29	HTB29	H	C	Colon	39
Colo201	CCL224	H	C	Colon	40
Colo205	CCL222	H	C	Colon	40
IA-Xs-SBR	CRL1677	R	C	Small intestine	41
NCI-H508	CCL253	H	C	Caecum	38
WiDR	CCL218	H	C	Colon	42
SW1417	CCL238	H	C	Colon	31
HCT-116	CCL247	H	A	Colon	43
LS180	CL187	H	C	Colon	44
SW948	CCL237	H	C	Colon	31
CMT-93	CCL223	M	A	Rectum	45
HCT-8	CCL244	H	C	Ileum	46
LS174T	CL188	H	C	Colon	44
HuTu80	HTB40	H	C	Duodenum	47, 48
T84	CCL248	H	A	Colon	49
SW1116	CCL233	H	C	Colon	31
NCI-H747	CCL252	H	C	Caecum	38

The gastrointestinal cell lines are ranked from the top to the bottom of the Table according to their increasing sensitivity to gastrin as is shown in Figures 1–7 and Table II. With respect to histopathology, N=normal, C=carcinoma, and A=adenocarcinoma. With respect to species, H=human, GP=guinea pig, R=rat, M=mouse, and G=goat.

factor present in the medium or secreted by autocrine or paracrine mechanisms, or both.

It should be emphasised that these antibodies would not cross the cell membrane and therefore, if there are intracrine mechanisms linked with some of these hormones, then these antibodies would not affect such a mechanism.

Cell proliferation was assessed by means of the colorimetric MTT assay.^{28–30}

Methods

CELLS AND MEDIUM

All the 27 gastrointestinal cell lines were obtained from the ATCC (Rockville, MD). Table I lists and describes the cell lines. The 27 cell lines were adapted to grow as monolayers cultured at 37°C in closed Falcon plastic dishes (Nunc, Poly Labo, Strasbourg, France) containing Eagle's minimal essential medium (Gibco, Cergy Pontoise, France) supplemented with 10% fetal calf serum (Gibco).

All the mediums were supplemented with a mixture of 0.6 mg/ml glutamine (Gibco), 200 IU/ml penicillin (Gibco), 200 µg/ml streptomycin (Gibco), and 0.1 mg/ml gentamycin (Gibco). The fetal calf serum was heat inactivated for one hour at 56°C.

EXPERIMENTAL SCHEDULE FOR CELL GROWTH ASSESSMENTS

Cell growth was assessed by means of the MTT assay as previously described³⁰ – that is, according to Mosman²⁸ with the modifications recommended by Carmichael *et al.*²⁹ Briefly, after the incubation of the cells for 96 hours in the various mediums, the culture medium was removed and replaced with 100 µl MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl

tetrazolium bromide, Sigma) at 1 mg/ml RPMI medium (Seromed, Berlin, Germany). The plates were incubated for three hours at 37°C and then centrifuged for seven minutes at 400 g. The medium was replaced with 100 µl dimethylsulphoxide (DMSO). The multiwells were shaken on a plate shaker for 10 minutes; they were then read on a Bio-Tek Instrument Microplate Reader (EL 308) using a test wavelength of 570 nm and a reference one of 630 nm.²⁸

The cells were incubated in an anti-hormone or anti-growth factor antibody-free medium, for 24 hours in each experimental condition to ensure good plating conditions.

In each experiment the various cell lines were incubated for 72 hours (after the 24 hour plating procedure) in either an anti-hormone or an anti-growth factor antibody-free medium, or both (control), or one supplemented either with 50 ng/ml (0.33 nM), 500 ng/ml (3.3 nM), 5000 ng/ml (33 nM), or 10 000 ng/ml (67 nM) of each of the four antibodies under study – that is, the anti-gastrin (polyclonal A568 rabbit antibody, Dakko, Trappes, France), the anti-EGF (monoclonal MAB126 mouse antibody, Euromedex, Schiltgheim, France), the anti-E₂ (monoclonal MAB1233 mouse antibody, Euromedex), and the anti-LHRH (polyclonal AB923 rabbit antibody, Euromedex) antibody.

All the assays were performed in sextuplicate.

STATISTICAL ANALYSES

The assessments of the cell proliferation (MTT test) are reported as means (SEM) statistically compared by means of the Fisher *F* test (one way variance analysis). Equality of variance was checked by the Bartlett test. Values of statistical significance represented in the Figures are: *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$. Correlations between results obtained by different tests were assessed by means of the non-parametric Kendall rank correlation test.

Results

The Figures show the anti-hormone or anti-growth factor antibody induced effects, or both on the proliferation of the 27 cell lines. In these Figures the mean value of the optical density measured in the control condition at the 96th hour of culture has been arbitrarily represented as equal to 0%. This value of 0% corresponds to the dark horizontal line that crosses all the graphs in Figs 1–7. The standard errors on the mean are not represented because they were too weak – that is, less than 3% compared with the mean values. The results relating to the MTT test are thus expressed as a gain or loss in the cell proliferation rate compared with the '0%' control value: this made an easy comparison possible between all the experimental conditions.

The 27 cell lines represented in Figs 1–7 are ordered according to their increasing sensitivity to the higher (10 000 ng/ml) dose

Figure 1: The determination of cell growth by means of the colorimetric MTT assay under five experimental conditions in the gastrointestinal SW403, AGS, SW1463, and CH1ES(NBL-8) cell lines. Table I gives the description of the cell lines. The five experimental conditions correspond either to the absence (control) or the presence of the anti-gastrin (G), the anti-epidermal growth factor (EGF), the anti-oestradiol (E_2), and the anti-luteinising hormone releasing hormone (LHRH) antibodies. Four concentrations were tested for each antibody – 10 000 (black bars), 5000 (shaded bars), 500 (hatched bars), and 50 (open bars) ng/ml. The mean value of the optical density measured in the control condition at the 96th hour of culture was arbitrarily defined as equal to 0% (dark horizontal line crossing the graph). The SEM of the control values are not represented because they were too weak and less than 3% when compared with the mean values. The mean (SEM) values were statistically compared by means of the Fisher F test (one way variance analysis). The values of statistical significance represented in the Figure are: *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$.

(black bars in Figs 1–7) of the anti-gastrin antibody. Thus, the SW403 cell line represented in the upper left hand section of the chart in Fig 1 corresponds to a cell line for which a very weak effect was seen in connection with the 10 000 ng/ml anti-gastrin antibody. This is in sharp contrast with the cell lines represented in Fig 7.

We have chosen to base our argument on the effect brought about by the maximum dose of the antibody tested here – that is, 10 000 ng/ml – because we consider that this is the dose

that eliminates the higher concentration of the hormone or growth factor under consideration. Thus, we estimate that it is this 10 000 ng/ml dose of the antibody that provides a more precise definition of the hormone sensitivity profile of the cell line considered.

Nevertheless, Figs 1–7 show that some cell lines (SW403, CoLo201, WiDR) exhibited a low degree of sensitivity, if any, to a given antibody at the highest dose tested, while a significant antibody induced effect was seen on the same cell lines at the lowest doses.

Figure 2: The determination of cell growth by means of the colorimetric MTT assay under five experimental conditions in the gastrointestinal SW48, DLD-1, GPC-16, and CoLo320DM cell lines. The legend is identical to that of Fig 1.

Figure 3: The determination of cell growth by means of the colorimetric MTT assay under five experimental conditions in the gastrointestinal LS123, SNU-C2B, HT-29, and CoLo201 cell lines. The legend is identical to that of Fig 1.

This apparent paradox is considered in the discussion.

Regarding Figs 1–7, it seems impossible to classify the 27 gastrointestinal cell lines under study into subgroups sharing a common hormone sensitivity profile. This is the reason why we decided to study the antibody induced effect at its highest dose to isolate a possible

logic behind the hormone sensitivity profile of these 27 cell lines. Table II reports the results where the 27 cell lines are ordered from the least sensitive (top of Table II) to the most (bottom) sensitive cell lines with respect to the anti-gastrin antibody at the 10 000 ng/ml dose as for Figs 1–7. In other words, Table II constitutes a summary of the data presented in

Figure 4: The determination of cell growth by means of the colorimetric MTT assay under five experimental conditions in the gastrointestinal CoLo205, IA-Xs-SBR, NCI-H508, and WiDR cell lines. The legend is identical to that of Fig 1.

Figure 5: The determination of cell growth by means of the colorimetric MTT assay under five experimental conditions in the gastrointestinal SW1417, HCT-116, LS180, and SW948 cell lines. The legend is identical to that of Fig 1.

Figs 1–7. The symbols used to characterise the anti-hormone or anti-growth factor antibody-induced effects on cell proliferation are as follows. When the antibody induced effect at the 10 000 ng/ml dose varied by less than 20% in relation to control, we indicate their effect by 0. When the effect was between –30 and –20% or +20 and +30% in relation to

control, we use the symbols – and +. When the effect was between –50 and –30% or +30 and +50%, the symbols are – – and + +. When the effects were <–50% or >+50%, the symbols are – – – and + + +. It seems that in terms of these arbitrarily chosen cut-off values, seven cell lines were gastrin insensitive or only marginally sensitive to gastrin (arbi-

Figure 6: The determination of cell growth by means of the colorimetric MTT assay under five experimental conditions in the gastrointestinal CMT-93, HCT-8, LS174T, and HuTu80 cell lines. The legend is identical to that of Fig 1.

Figure 7: The determination of cell growth by means of the colorimetric MTT assay under five experimental conditions in the gastrointestinal T84, SW1116, and NCI-H747 cell lines. The legend is identical to that of Fig 1.

trary value=0), seven cell lines were weakly sensitive to gastrin (arbitrary value=– or +), while the remaining 13 ones were highly gastrin sensitive (arbitrary value=–, ––, ++, or +++). In sharp contrast with what was seen with respect to gastrin, 25 of 27 cell lines were highly sensitive to EGF (Table II). As for the anti-E₂ and anti-LHRH antibodies, there were 12 and 16 cell lines respectively,

which were highly sensitive to these hormones.

Table II shows that the distribution over the four antibodies of the arbitrary values defined above seems to be random. In fact, as four distinct antibodies were used in this study, there are 16 combinations that make a hormone sensitive phenotype of the cell line possible. To caricature the antibody induced effects and to try to find a law governing their action at cell proliferation level, we only considered their effects in terms of absolute values – that is, independently of the fact that such an effect stimulated or inhibited cell proliferation. On the other hand, we only retained those effects whose arbitrary values equalled the ––, –––, ++, and +++ cut off values recorded at the 10 000 ng/ml dose. This was done because it was at this 10 000 ng/ml dose that the greatest biological hormone or growth factor activity was inhibited, and as a consequence it was at this dose that the most accurate mirror image was obtained of what the influence of the hormone or the growth factor at its physiological dose might have been.

Sixteen theoretical phenotypes of hormone sensitivity can be defined in terms of the four hormones or growth factors under study. These phenotypes are defined as follows. The cell line that exhibited no response to the four antibodies at their highest dose was labelled HI – that is, hormone insensitive (phenotype no 1). The cell line that was sensitive to only one hormone or growth factor was defined as either gastrin, EGF, E₂, or LHRH sensitive (phenotypes no 2–5). The cell line was sensitive to two of the four antibodies tested. Six combinations exist – that is, sensitive to gastrin and EGF (or to EGF and gastrin, phenotype no 6 defined as gastrin/EGF),

TABLE II Summary of the data relating to the influence on the proliferation of the 27 gastrointestinal cell lines under study of the anti-G, the anti-E₂, the anti-EGF, and the anti-LHRH antibody at the 10000 ng/ml dose

Name	Anti-X antibody induced effect				Resulting phenotype
	G	EGF	E ₂	LHRH	
SW403	0	0	0	---	LHRH
AGS	0	---	---	---	EGF/E ₂ /LHRH
SW1463	0	---	+	---	EGF/LHRH
CH1ES (NBL-8)	0	---	0	---	EGF
SW48	0	---	0	---	EGF/LHRH
DLD-1	0	---	---	0	EGF/E ₂
GPC-16	0	---	---	---	EGF/E ₂
Colo320	---	---	---	---	EGF/E ₂ /LHRH
LS123	---	---	---	---	EGF/E ₂ /LHRH
SNU-C2B	---	---	---	---	EGF/E ₂ /LHRH
HT-29	---	---	0	0	EGF
Colo201	---	---	---	---	EGF/E ₂ /LHRH
Colo205	---	---	---	---	EGF/E ₂ /LHRH
IX-Xs-SBR	---	---	---	0	E ₂
NCI-H508	---	---	0	+	G/EGF
WiDR	---	---	0	0	G/EGF
SW1417	---	---	0	---	G/EGF
HCT-116	---	---	---	---	G/EGF/E ₂ /LHRH
LS180	---	---	0	---	G/EGF/LHRH
SW948	---	---	++	---	G/EGF/E ₂ /LHRH
CMT-93	---	---	---	---	G/EGF/E ₂ /LHRH
HCT-8	---	---	0	---	G/EGF
LS174T	---	---	0	0	G/EGF
HuTu80	---	---	---	---	G/EGF/E ₂ /LHRH
T84	---	---	0	---	G/EGF/LHRH
SW1116	---	---	0	0	G/EGF
NCI-H747	---	---	0	---	G/EGF/LHRH

We have divided the antibody into four groups on the basis of the increase in their effect on NSCLC cell proliferation. When the antibody induced effect varied by less than 20% in relation to control, we indicate the effect by 0. When the effect is between –30 and –20% or +20 and +30% in relation to control, we use the symbols – and +. When the effect is between –50 and –30% or +30 and +50%, the symbols are –– and ++. When it is <–50% or >+50%, the symbols are ––– and +++.

Figure 8: Relation between the anti-gastrin (G) and anti-EGF antibody induced effects (top) and between the anti-E₂ and anti-LHRH antibody induced ones (bottom). The black dots represent the 10 000 ng/ml dose and the open dots represent the 500 ng/ml one. The values relating to the increment of proliferation come from Figs 1–7.

gastrin and E₂ (phenotype no 7), gastrin and LHRH (phenotype no 8), EGF and E₂ (phenotype no 9), EGF and LHRH (phenotype no 10), and E₂ and LHRH (phenotype no 11). The cell line was sensitive to three of the four antibodies. Four phenotypes exist – that is, gastrin/EGF/E₂ (phenotype no 12), gastrin/EGF/LHRH (phenotype no 13), EGF/E₂/LHRH (phenotype no 14), and gastrin/E₂/LHRH (phenotype no 15). Lastly, only one combination defined the phenotype describing the fact that the cell line was sensitive to the four hormones or growth factors. This phenotype was defined as

gastrin/EGF/E₂/LHRH (phenotype no 16). Random probability dictates that we should have observed at least one cell line out of the 27 associated with one of each of the 16 theoretical phenotypes. Table II shows, however, that of the 16 theoretical phenotypes only some were experimentally observed. Furthermore, Table II also shows that some of these experimentally observed phenotypes were seen more frequently than others. The theoretical phenotypes not experimentally seen were the HI, the gastrin, the gastrin/E₂, the gastrin/LHRH, the E₂/LHRH, the gastrin/EGF/E₂, and the gastrin/E₂/LHRH. In sharp contrast, the experiments showed that of the remaining nine phenotypes three were seen predominantly. These were the gastrin/EGF, the EGF/E₂/LHRH, and the gastrin/EGF/E₂/LHRH. The other six phenotypes, the EGF, the E₂, the LHRH, the EGF/E₂, the EGF/LHRH, and the gastrin/EGF/LHRH, were characterised between one and three cell lines of the 27 under study.

This experimental absence of some theoretical phenotypes and the presence of some other predominant ones led us to try to find a possible statistically significant relation between the combined effect of some of the hormones or growth factors under study. Figure 8 and Table III shows the results.

Table III shows that the anti-gastrin antibody induced effect on the proliferation of the 27 cell lines at the 10 000 ng/ml and 500 ng/ml doses was highly correlated with those of the anti-EGF antibody. A statistically significant effect was also seen with the 5000 ng/ml dose but not with the 50 ng/ml one (data not shown). In the same way, the anti-E₂ antibody induced effect on the proliferation of the 27 cell lines correlated significantly with that of the anti-LHRH effect. This effect was more noticeable at the 5000 (data not shown) and 500 ng/ml doses than at the 10 000 ng/ml one. No statistically significant relation was seen at the 50 ng/ml dose (data not shown).

Table III shows that, in sharp contrast with what is described before, no statistically significant relation was seen between the other combinations – that is, the anti-gastrin antibody compared with the anti-E₂ or anti-LHRH ones on the one hand and the anti-EGF antibody compared with the anti-E₂ and anti-LHRH ones.

Figure 8 illustrates the pattern of such a significant relation between the anti-gastrin and anti-EGF antibodies and between the anti-E₂ and anti-LHRH antibodies. The black dots correspond to the antibody induced effects at the 10 000 ng/ml dose while the open

TABLE III Statistical *p* value from Kendall rank correlation test performed on the proliferation values obtained after adding the different anti-hormone or anti-growth factor antibody in the culture medium

	Dose (10 000 ng/ml)			Dose (500 ng/ml)		
	Anti-EGF antibody	Anti-E ₂ antibody	Anti-LHRH antibody	Anti-EGF antibody	Anti-E ₂ antibody	Anti-LHRH antibody
Anti-G antibody	p=0.0002	p=0.0975	p=0.2740	p<0.0001	p=0.8780	p=0.4962
Anti-EGF antibody	–	p=0.8128	p=0.6346	–	p=0.3840	p=0.2484
Anti-E ₂ antibody	–	–	p=0.0518	–	–	p=0.0032

dots correspond to the effects recorded at the 500 ng/ml dose. It seems that such relations are linear and positive and are more pronounced between the anti-gastrin and anti-EGF antibodies than between the anti-E₂ and anti-LHRH ones.

Discussion

In a study (unpublished data) we describe the influence of various anti-hormone or anti-growth factor antibodies on the proliferation of two human neoplastic colorectal cell lines, the LoVo and HCT-15 cells. The data show that of the 19 antibodies tested, 10 modified the *in vitro* proliferation of these two cell lines very significantly. These 10 antibodies were the anti-insulin like growth factor I, the anti-transforming growth factor alpha, the anti-basic fibroblast growth factor, the anti-carcinoembryonic antigen, the anti-prolactin, the anti-platelet derived growth factor, the anti-gastrin, the anti-EGF, the anti-E₂, and the anti-LHRH antibodies. As for the remaining nine antibodies in this study (the anti-transforming growth factor beta, the anti-tumour necrosis factors alpha and beta, the anti-bombesin, the anti-nerve growth factor, the anti-substance P, the anti-secretin, the anti-somatostatin, and the anti-vasoactive intestinal peptide antibodies), we were not able to show any really significant influence on LoVo and HCT-15 cell proliferation.

To test the clinical relevance of these data we decided to continue our experiment with an investigation into the effect on a large series of gastrointestinal cell lines of anti-hormone or anti-growth factor antibodies known to have a considerable influence on the proliferation of the LoVo and the HCT-15 cell lines. This is the aim of this study, which characterises the influence of the anti-gastrin, anti-EGF, anti-E₂, and anti-LHRH antibodies on the proliferation of 27 gastrointestinal cell lines.

The methodology used here is unusual. Indeed, it was the anti-hormone or anti-growth factor antibody rather than the hormone or growth factor itself that was used to characterise the hormone sensitivity profile of the gastrointestinal cell line under consideration. We used this methodology because our laboratory has been able to observe some very considerable interference in the field of cell proliferation between the effect of the hormone added exogenously to the culture medium and that of the hormone existing endogenously in the same medium. This finding, made over a number of years, is based on a very large number of experimental models. Our present argument is wholly based on the opinion that the addition of the antibody to the culture medium will inhibit the biological activity of the hormone or the growth factor targeted and that the most effective inhibition of this biological activity is obtained at the highest concentration used here, namely 10 000 ng/ml. In other words, we believe that a concentration of 10 000 ng/ml provides the best mirror image of what the influence of the hormone or growth factor would have been at

an equivalent concentration. We could not test any higher antibody concentrations because of the cost of the experiments.

It should also be emphasised that we preferred to use the anti-hormone or anti-growth factor antibody rather than the hormone or growth factor itself because more information becomes available in this way. Indeed, a positive effect of hormones or growth factors added to the medium is evidence of sensitivity of the tumour cells to the hormone or growth factor tried, but it does not provide any information on the possible action of the same hormone or growth factor on cultures growing in unsupplemented medium. The influence of neutralising antibodies on growth of the culture is proof that the cell lines were already stimulated by the corresponding hormone or growth factor, either present in the fetal calf serum used in the medium, or secreted by an autocrine or paracrine mechanism, or both by the tumour cells themselves.

It should also be emphasised that this study assessed the proliferation rate by means of the recently criticised⁵⁰⁻⁵² colorimetric assay.²⁸⁻³⁰ We maintain that we really did measure the cell proliferation rate by means of the MTT test because we carried out the appropriate controls. Indeed, in three distinct studies (reference 12 and two manuscripts submitted for publication) we compared the data obtained by means of two different tests with those obtained by means of the MTT test. These two tests entailed the tritiated-thymidine incorporation into the genomic material⁵³ and direct cell nuclei counting.^{12 54} The results showed identical results with the three tests with respect to the inhibitory influence on the proliferation of the LoVo and HCT-15 cell lines of the anti-carcinoembryonic, the anti-EGF, and the anti-LHRH antibodies.

These results show that it was the EGF (93%) and not the gastrin (48%) that significantly influenced the highest number of gastrointestinal cell lines under study. Also the E₂ and LHRH significantly influenced the proliferation of about half of the 27 cell lines in terms of cell proliferation. None of the gastrointestinal cell lines was simultaneously insensitive to the four hormones or growth factors. These data are thus corroborated by those of Frucht *et al.*²⁷ who report that a total of 27 receptors out of a possible 120 (23%) occurred on 10 gastrointestinal cell lines, a fact which shows in our opinion that the presence of receptors for gastrointestinal hormones and neurotransmitters in human colon cancer cell lines is not an uncommon occurrence. Both our data and those of Frucht *et al.*²⁷ clearly show that the gastrin does not play an important part with respect to the regulation of gastrointestinal cell line proliferation.

This study shows that there were 16 theoretical hormone sensitive phenotypes that could exist given the fact that four hormones or growth factors were used; of these only nine were present, and of these nine, three predominated. These facts strongly suggest that coregulating phenomena with respect to gastrointestinal cell proliferation occur

between the four hormones or growth factors under study. By way of an example, none of the 27 gastrointestinal cell lines under study seemed to be sensitive to the gastrin only, as was also the case with the E_2 +LHRH. Moreover, the gastrin/ E_2 , the gastrin/LHRH, and the gastrin/ E_2 /LHRH do not exist. Everything suggests that the gastrin and E_2 +LHRH would act as independent regulators of the *in vitro* gastrointestinal cell proliferation. In addition to these two independent regulators, the EGF seems to constitute a third regulator that is not independent of the other two, but more the dominant partner. However, the gastrin/EGF/ E_2 phenotype does not exist. It thus seems impossible to see a noticeable and concomitant gastrin and E_2 induced stimulation of the gastrointestinal cell proliferation. These two hormones seem to be mutually exclusive with respect to their action on the gastrointestinal cell proliferation. This feature might be related to up and down regulation phenomena exerted by the gastrin and E_2 at the level of their secretion or receptor expression, or both.^{55 56}

While the results show that the effects of certain hormones or growth factors can be correlated with the proliferation of the neoplastic gastrointestinal cells, they also raise the problem of the possible effectiveness of mono-hormonotherapy on this type of cancer. In fact, antagonising the biological activity of a hormone by the use of an antagonist is not wholly inconceivable. This is true for tamoxifen and oestradiol in the case of breast cancers. These results strongly suggest that, in the case of the projected treatment of colorectal cancer by hormonotherapy, there would be an immediate need for poly-hormonotherapy including at least gastrin, oestradiol, and LHRH antagonists because otherwise the hormone whose effect had not been antagonised at proliferation level might replace the other one. In the same way, it seems that the use of a gastrin antagonist alone would not be enough to inhibit the proliferation of neoplastic gastrointestinal cells sensitive to it as the EGF is more than likely to take over. Experiments are being carried out in the laboratory to investigate the effect of various combinations between the anti-gastrin, the anti- E_2 , and the anti-LHRH antibodies on the gastrointestinal cell line proliferation.

The results described concern four hormones or growth factors, of which three are well known for influencing gastrointestinal cell proliferation. These are the gastrin,¹⁻¹² the E_2 ,¹⁹⁻²¹ and the EGF.^{22 23} To our knowledge we were the first to show that the LHRH can also significantly influence the proliferation of colorectal cells,²¹ a feature that is confirmed by this study on a large series of gastrointestinal cell lines.

As Table I shows, the 27 gastrointestinal cell lines did not exhibit the same histopathological characteristics or origin. We tested whether these differences in histopathological (colorectal compared with non-colorectal, carcinoma compared with adenocarcinoma, etc) characteristics and origin (human compared

with non-human) could influence the results. No statistically significant results were obtained (data not shown).

The results show that most of the anti-hormone or anti-growth factor antibodies induced a dose dependent effect on the gastrointestinal cell proliferation. This was the case with respect to the anti-EGF antibody on the GPC-16 cell line (Fig 2) and to the anti-LHRH antibody on the NCI-H508 one (Fig 4). In contrast, some intermediate situations were also seen. For example, this was the case with respect to the SW403 cell line, where the anti-gastrin, the anti-EGF, and the anti- E_2 antibodies induced an increase and not a decrease in cell proliferation (Fig 1). This was also seen with respect to the effect of the anti- E_2 antibody on SW1463 (Fig 1) and LS180 (Fig 5) cell proliferation. It was also seen that a given antibody induced a potent inhibition of cell proliferation at a high dose but a stimulation of the same cell proliferation at a lower one. This was the case with the effects of the anti-LHRH antibody on SW403 cell proliferation (Fig 1), of the anti-EGF antibody on CoLo320DM proliferation (Fig 2), and of the anti- E_2 antibody on the CoLo205 (Fig 4).

We think that such antibody induced rebound effects might be partly explained by the fact that the hormones or the growth factor may exert a dual influence on the proliferation of some cancers. This dual influence corresponds to a stimulating influence on cell proliferation accompanied by an increase in mitoses, and to an inhibitory one accompanied by an increase in apoptosis. Such dual hormone or growth factor induced effects on cell proliferation are described in several experimental models related to the liver,⁵⁷ the MXT mouse mammary carcinoma,⁵⁸ the uterus,⁵⁹ and the prostate.⁶⁰

In conclusion, we believe that when the antibody brings about a significant decrease in cell proliferation when tested at the highest concentration, this corresponds to the elimination of the mitogenic activity of the hormone or the growth factor. In contrast, we believe that when a lower concentration of this antibody brings about an increase in cell proliferation, this corresponds to the elimination of a part of the hormone or growth factor induced apoptosis related cell death effect. As the cell lines proliferate very rapidly *in vitro* (and never regress spontaneously), it is logical for us to have seen that while the mitogenic biological activity of a hormone or growth factor is associated with large amounts of the antibody, the biological activity connected with the apoptosis entails smaller amounts. We are in the process of testing this hypothesis by means of biochemical (endonuclease activation) and electron microscopy (search for apoptotic bodies) experiments.

These data characterised the influence of the anti-gastrin, the anti-EGF, the anti- E_2 , and the anti-LHRH antibodies on the proliferation of 27 gastrointestinal cell lines and show that it was the anti-EGF neutralising antibody that had the greatest (93%) and the

most noticeable influence on the gastrointestinal cell line proliferation. In contrast, the anti- E_2 , the anti-gastrin, and the anti-LHRH neutralising antibodies had a real influence on only about half of the gastrointestinal cell proliferation. Thus, the gastrin was not the major regulator of the gastrointestinal cell line proliferation. The anti-gastrin and anti-EGF antibody induced effects on the 27 gastrointestinal cell line proliferation were significantly correlated, as was also the case for the anti- E_2 and anti-LHRH antibody induced effects. Lastly, none of the gastrointestinal cell lines was totally hormone insensitive and in view of the fact that four anti-hormone or anti-growth factor antibodies were used in this study, 16 theoretical hormone sensitive phenotypes were possible but only nine were experimentally seen. Of these nine phenotypes, three were predominant. These were defined as the gastrin/EGF, the EGF/ E_2 /LHRH, and the gastrin/EGF/ E_2 /LHRH and showed that the gastrointestinal cell lines exhibited an equal degree of sensitivity to gastrin+EGF, EGF+ E_2 +LHRH, and to gastrin+EGF+ E_2 +LHRH.

Additional experiments are currently under way in the laboratory to characterise the biochemical transduction pathway used by these various anti-hormone or anti-growth factor antibodies.

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