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COMMENTARY

EFFECTS OF RETINOIDS ON GROWTH AND DISSEMINATION OF MALIGNANT TUMOURS: IMMUNOLOGICAL CONSIDERATIONS

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Vitamin A (retinol) and many of its natural and synthetic analogues ("retinoids", see Fig. 1) have been found to play a critical role in the normal growth and differentiation of a variety of tissues, and in this connection their influence in malignant disease, which may be considered to be a state of abnormal growth and/or differentiation, has received much attention. Since the earliest observations in the 1920s demonstrated that a Vitamin A deficient diet could lead to the development of metaplasias [1], papillomas, and ultimately squamous cell carcinomas in rodents [2], much subsequent work has concentrated on the role of retinoids in modification of the carcinogenic process. The prophylactic effects of retinoids in the prevention of tumours induced by chemicals, radiation and viruses in both epithelial and mesenchymal tissues are now well documented and have been comprehensively reviewed recently [3–10]. However, the current major problem in clinical oncology is the management not of benign lesions, or carcinomas *in situ* which respond well to therapeutic intervention, but of malignant tumours which have progressed to become invasive and metastatic. In this article, I propose to focus on the less well-defined effects of retinoids on established tumour growth and metastasis, and to explore in particular their interactions with host tissues which may determine the *in vivo* response.

Effects of retinoids on tumour growth in experimental animals

In contrast to the well-documented prophylactic effects of retinoids in retarding, preventing, or in some cases reversing carcinogenesis, reports regarding the ability of retinoids to inhibit the growth of malignant transplantable tumours are often conflicting. Bollag and coworkers [7, 11] tested several conventionally used transplantable rodent tumours (including Ehrlich carcinoma, sarcoma 180, L1210 leukaemia, Walker 256 carcinoma, B16 melanoma, and Lewis lung carcinoma) for their responses to two aromatic retinoic acid analogues TMMP-EE and TMMP-EA (see Fig. 1), and obtained no growth inhibition in any instance. Similar findings were reported for TMMP-EE by Ito [12] using V × 2 and V × 7 transplantable carcinomas in rabbits, although Shope virus-induced papillomas similar to those from which they arose were strongly growth inhibited (with up to 60% regressions) by the same retinoid. Retinoic acid and retinyl palmitate were also shown to have no growth inhibitory effects against L1210 leukaemia [13, 14], Lewis lung carcinoma [15, 16], P388 mastocytoma [17], a Morris hepatoma in the rat [18], or syngeneic weakly immunogenic sarcomas in mice [19–22].

These negative results are nonetheless informative: one common feature of these "non-responder" tumours is that they have been maintained by serial transplantation in some cases for up to 40 years. Many have lost all the characteristics of the original primary tumour and, in particular, any immunogenicity originally present would be significantly diminished; this supposition is borne out by the fact that such tumours are generally readily transplantable in outbred (i.e. genetically heterogeneous) animals and a variety of allogeneic (i.e. histoincompatible) strains. Thus, one possibility that must be examined is whether the responsiveness of a transplanted tumour may depend on its ability to evoke an immune response in the host which can be augmented by retinoids via their immunoadjuvant properties [23, 24].

In this regard the literature reveals that growth inhibitory effects of retinoids have regularly been obtained in systems where an immune response to the tumour was demonstrated, or where tumour immunogenicity can be inferred, e.g. by virtue of their induction by certain chemicals (e.g. polycyclic hydrocarbons), particular viruses (e.g. SV40, M-muSV) or by their transfer to histoincompatible hosts (see Table 1) [19, 20, 22, 25–31]. For example, Deneufbourg was able to increase the regression rate of sarcoma J by TMMP-EE; this tumour is subject to "unique immune control mechanisms" in syngeneic mice [28]. Patek *et al.* [22] obtained significant growth inhibition by retinoic acid (RA) with S-91 melanoma in allogeneic mice (this effect was lost if they were immunosuppressed by thymectomy plus irradiation, (TX1), and with L-33 tumour which expresses anti-

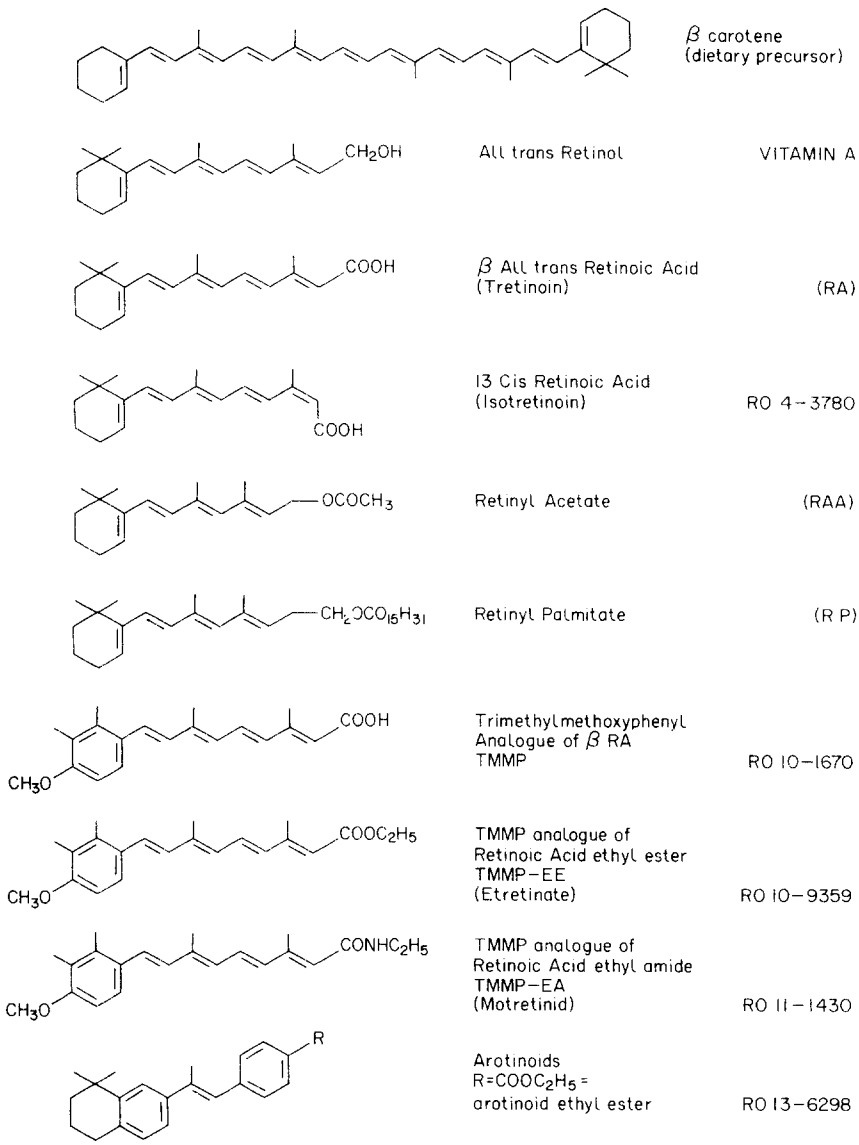

Fig. 1. Retinoid chemistry.

gens associated with SV-40 virus transformation in syngeneic mice. It was also shown that S-91 melanoma was strongly suppressed by retinyl palmitate in allogeneic mice but, again, not in animals immunosuppressed by anti-lymphocyte serum, and not in F_1 hybrid mice which shared H-2 antigens with the tumour [19, 20]. These results are significant since S-91 melanoma cells are extremely sensitive to retinoids *in vitro* [32], and it has been suggested that their growth inhibition *in vivo* is due to a direct effect of retinoids on tumour cell proliferation. However, the lack of effect of retinoids in histocompatible mice, or in immunocompromised allogeneic hosts, suggests that *in vitro* responsiveness does not necessarily predict *in vivo* sensitivity, and where tumour growth inhibition *in vivo* is obtained, it may depend on a quite different mechanism requiring interaction with an immunocompetent host.

In an attempt to clarify further the potential of retinoids to control the growth of transplantable tumours *in vivo*, and to investigate systematically the contribution of direct and indirect modes of action, we chose to test *in vitro* and *in vivo* the retinoid response of a variety of tumours (chemically induced, virally induced, and "spontaneous") of recent origin and defined immunogenicity [33–36]. We selected TMMP-EE, an aromatic analogue with a ten times more favourable therapeutic ratio than retinoic acid [6], to minimize toxic side effects *in vivo*, and its active derivative (TMMP) [12] for use *in vitro*. All the tumours arose or were induced in our own inbred strains of mice and were used during their first twenty transplant generations. All studies were carried out in mice of the same strain and sex as the primary tumour host to eliminate spurious immunogenicity due to H-2 or H-Y incompatibility.

The results obtained are summarized in Table 2. Four out of six fibrosarcomas, one of three squamous

Table 1. Tumour immunogenicity

1. Postive	e.g.
(a) Chemical carcinogens tend to produce tumours with unique antigens; they can be potent immunogens	DM6 FS6
(b) Transformation with certain viruses (e.g. SV40, M-muSV) leads to expression of strongly immunogenic surface antigens	L-33
(c) Some "spontaneous" tumours express weak or strong immunogens; they may be sufficient to induce regressions in syngeneic hosts	ST3 SaJ
(d) Any tumours transferred to histo-incompatible hosts will evoke immune responses to H-2 antigens (unless serially transplanted as allografts—see 2b)	S-91
2. Negative/Undetectable	e.g.
(a) Many "spontaneous" tumours do not evoke detectable transplantation immunity	SM1 ST4
(b) Immunogenicity is reduced by serial transplantation; the rate of loss may vary [33] Tumour-associated and/or H-2 antigens can be lost	DM6 FS6 3LL
(c) No antigens, however strongly immunogenic, can evoke specific immunity in immunosuppressed recipients	FS6 DM6 S-91
(d) Non-productive transformation with certain viruses (e.g. KiMSV, mMTV, Harvey sarcoma virus) leads to little or no immunogenicity	MT1 KA31 HT3-2.1

cell carcinomas and one of five mammary adenocarcinomas responded to TMMP-EE treatment *in vivo* by reduced growth rates (first detectable after 8–10 days) and in some cases (e.g. FS6) by complete regressions. The magnitude of the response was directly related to the immunogenicity of a particular tumour, and eight tumours that failed to respond to the retinoid did not evoke detectable transplantation immunity in syngeneic recipients. When the host response to tumour antigens was abolished by any of three different immunosuppressive procedures (500 rads whole body X-irradiation; TXI, Cyclosporin A treatment), the tumour response to retinoids was abrogated. Also, in two cases, FS6-FS6M1 sarcomas and DM6 carcinomas, the ability of the tumours to evoke host immunity diminished during serial transplantation; in both cases this was accompanied by a loss of response to retinoids *in vivo*.

The growth rates of most of the tumours *in vitro* were unaffected by the presence of TMMP at 10^{-6} or 10^{-8} M concentrations. Growth inhibition of MT3 carcinoma was obtained at both concentrations, although this tumour was unresponsive to retinoid *in vivo*. Growth inhibition of MT1 carcinoma was obtained with 10^{-6} M retinoid using a cloned cell line of epithelioid morphology, but not using a cell line of fusiform morphology derived from the same parent tumour [34]; neither line was responsive to retinoids *in vivo*.

No evidence of increased differentiation in response to retinoids was obtained; the morphology of tissue cultured cells was not altered; and the frequency of "dome" formation in the mammary carcinomas and of keratin production in the squamous cell cultures was not affected significantly. Histological sections of tumours grown in retinoid-treated mice did not give any evidence of increased differentiation, but a more pronounced lymphoreticular "cuff" around regressing tumours was a common feature, and enzyme-disaggregated tumours from retinoid-treated mice were found to contain a significantly higher proportion of host mononuclear cells.

Effect of retinoids on spontaneous metastasis of experimental tumours

Very few studies have attempted to investigate systematically the effects of retinoids on the dissemination of cells from a primary tumour and their colonisation of distant sites, although it would seem to be a neglected and possibly fruitful area of investigation. Whether acting by inhibition of cellular proliferation, induction of differentiation or augmentation of host anti-tumour defence mechanisms, micrometastases might present as accessible a target as early primary lesions.

Ito, in his study on the effects of TMMP-EE on rabbit carcinomas [12], noted a reduction in the number and size of pulmonary metastases in retinoid-treated hosts (in the absence of effects on the primary tumours), although no data were given, and the high dose of retinoid used (200 mg/kg) caused severe hypervitaminosis A. In contrast Weiss and Holyoke [39] described an increased frequently of lung metastases from a spontaneous mammary carcinoma in mice treated with retinyl palmitate. However, this study also suffered from the limitations that growth of the local tumours was very variable, and the mice again exhibited severe symptoms of retinoid toxicity. Morré and colleagues [40, 41] reported that, when rats were injected with a metastatic N-2 fluorenyl-acetamide-induced hepatocellular carcinoma 2 weeks after initiation of dietary regimens containing either zero, adequate or excess Vitamin A, the incidences of metastases were 60, 75 and 0%, respectively, suggesting that dietary supplements of Vitamin A can abolish spontaneous metastases. Again, we are given no data on the response of the primary

Table 2. Effect of retinoid on growth (*in vitro* and *in vivo*) of syngeneic transplantable tumours

Tumour	Immunogenicity/origin*	Effects *in vivo*†	Effects *in vitro*‡§
FS6 sarcoma	+++ (chemically induced) − (immunosuppressed host)	80–90% Complete regressions [35, 37, 38] No effect [37, 38]	No change in morphology or growth rate
FS29 sarcoma	++ (chemically induced)	Reduced growth rate [37, 38]	None detectable
FS6M1 sarcoma	± (lost on transplantation)	No effect with standard protocol [35, 37, 38] Pretreatment reduced growth rate§	None detectable
FS19 sarcoma	++ (chemically induced)	Reduced growth rate [37, 38]	Not tested
FS12 sarcoma	+ (chemically induced)	Slightly reduced growth rate§‖	None detectable
FS13 sarcoma	± (chemically induced)	No effect [37, 38]	None detectable
DM6 p10 carcinoma	+++ (chemically induced)	Reduced growth rate [37, 38]	No change in growth rate or keratin production
p20	− (immunosuppressed host) ± (lost on transplantation)	No effect [37, 38] No effect [38]	
DM2 carcinoma	± (chemically induced)	No effect [38]	Not tested
CA1 carcinoma	± (chemically induced)	No effect [38]	Not tested
SM1 carcinoma	± ("spontaneous")	No effect [38]	Not tested
MT1 carcinoma	± (muMTV induced)	No effect [38]	Some clones growth inhibited, some not, no increase in "dome" formation
MT3 carcinoma	± (muMTV induced)	No effect [38]	Growth inhibition, no increase in "dome" formation
ST3 carcinoma	+ ("spontaneous")	Slight growth inhibition [38]	None detectable
ST4 carcinoma	± ("spontaneous")	No effect [38]	Not tested

* Immunogenicity = relative ability specifically to increase the TD_{50} in pre-immunized syngeneic recipients. Key: (±) <1 log increase in TD_{50}; (+) 1–2 logs; (++) 2–3 logs; (+++) >3 logs; and (−) abolished by immunosuppression.

† *In vivo* retinoid treatment: 40 mg/kg TMMP-EE ("Tigason") in olive oil by oral intubation, 5 days/week beginning normally on day of tumour inoculation (day 0) and continuing until excision, or for 21–28 days if regression or growth delays occurred. In some cases treatment was initiated 18 days before tumour implantation. This dose was chosen to give serum concentrations of TMMP similar to those used *in vitro*.

‡ *In vitro* retinoid treatment: 10^{-6} or 10^{-5} M TMMP daily for 7–10 days; light was excluded.

§S. A. Eccles, S. C. Barnett and P. Alexander; and ‖S. A. Eccles, H. P. Purvies, S. C. Barnett and P. Alexander, *Cancer Immunol. Immunother.*, in press (1985).

tumours to these regimens, although the growth of other (non-metastatic) hepatomas was inhibited by either Vitamin A deficiency or excess.

It is important that the possible therapeutic effects of retinoids on metastasis be sought in animals not suffering severe toxicity, and in which the growth of the local tumours is taken into account: apparent inhibition of metastases may merely be due to the propensity of tumours to grow less well in poorly-nourished or sick animals [14, 42], and since the probability of metastasis is linked to development of the primary lesion, a growth inhibition of the latter would inevitably lead to an apparent reduction in metastatic disease. This is illustrated by the work of Kurata and Mickshé [15] investigating the combined antitumour effects of BCG and retinyl palmitate. The Lewis lung carcinoma responded to this treatment by reduced growth rate and, if mice were killed at 28 days, the mean number of lung metastases was 7.05 compared with 59.3 in the controls, and some treated animals had no discernible lung tumour. However, if the observation period was prolonged, all mice eventually died of lung metastases, showing that no real control of disseminated disease had been achieved.

Some experimental results from our laboratory are summarised in Fig. 2. The tumours are the same as those illustrated in Table 2. TMPP-EE was found not to inhibit metastasis of non-immunogenic tumours (e.g. SM1, DM6 p20, CA1, MT3), but significantly decreased the incidence of secondary disease of moderately immunogenic tumours (e.g. DM6 p7 and p10, and FS29). In many cases lymphatic metastasis, which was a common occurrence in untreated animals, was completely abolished by TMMP-EE. Metastasis was also absent in retinoid-treated animals bearing the strongly immunogenic tumours FS6, FS19 and DM6 p5, but since the incidence in control animals was only 10–20% (and most FS6 tumours regressed completely) this difference did not achieve statistical significance. These results are comparable with those reported by Tannock *et*

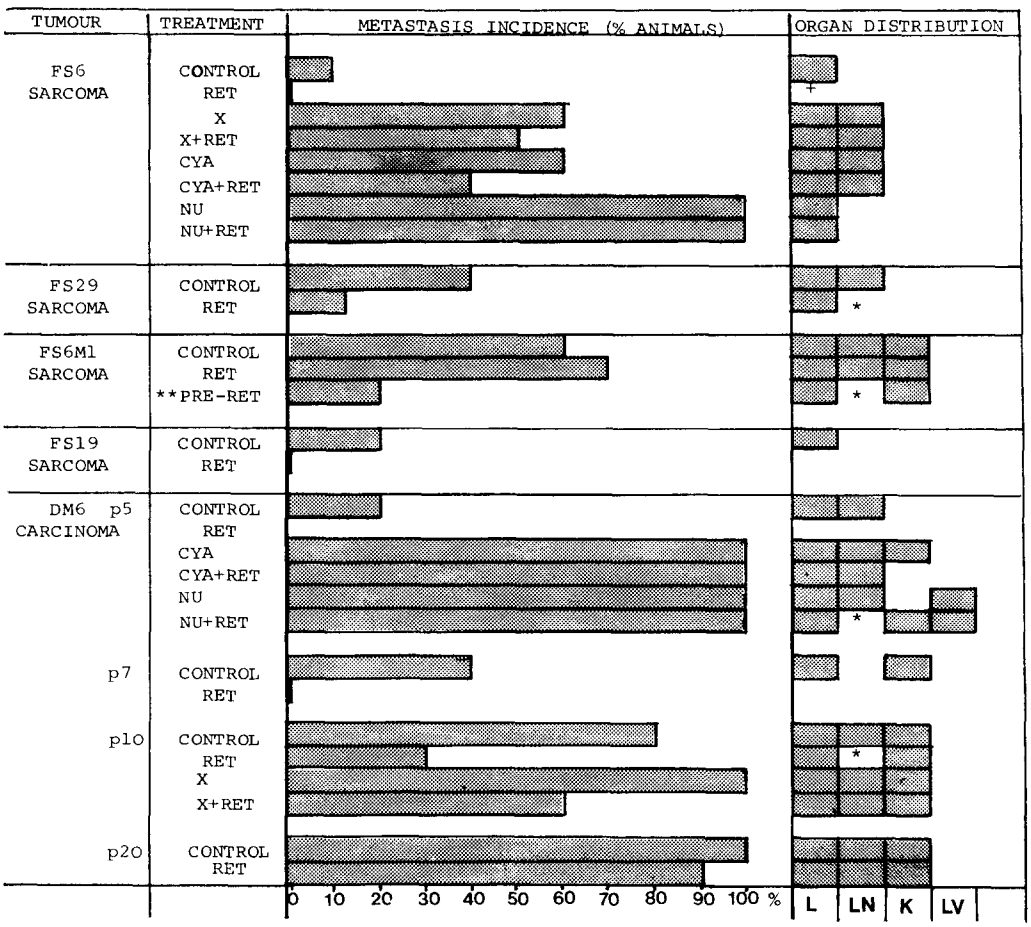

Fig. 2. Effect of TMMP-EE and host immunocompetence on spontaneous metastasis of syngeneic tumours. All tumours were grown s.c. and (except regressor FS6) excised at 8–10 mm mean diameter. Animals were observed for up to 1 year for development of metastases. Key: (+) 80% regressions; (*) LN metastases abolished; (**) Retinoid treatment initiated 18 days before tumour inoculation; all other treatments began on day 0; L = lung; LN = lymph node; K = kidney; LV = liver; X = 500 rads whole body X-irradiation; NU = congenitally athymic (nu/nu) mice; and CYA = Cyclosporin A, 200 mg/kg/day. No inhibition of metastasis was obtained for tumours FS12, FS13, MT1, MT3, ST4, CA1, and DM2 (Ref. 38 and S. A. Eccles *et al.*, *Cancer Immunol. Immunother.*, in press (1985)) (data not shown).

al. [43] where local tumour control was achieved by irradiation rather than surgery. The metastatic incidence of a moderately immunogenic carcinoma was reduced from 56 to 29% by Vitamin A treatment; the incidence of metastasis from a strongly immunogenic sarcoma was too low to determine any benefit.

Note also (Table 2) that with serial transplantation the incidence of metastasis of DM6 carcinoma gradually increased from 20% at passage (p) 5 to 100% at p20, and that the inhibitory effect of retinoids on spontaneous metastasis was concomitantly reduced. In three categories of immune-deprived mice (congenitally athymic nu/nu; sublethally irradiated; and Cyclosporin A treated), the incidence of spontaneous metastasis was increased significantly, and in these animals the retinoid had a markedly reduced therapeutic benefit.

The effects of TMPP-EE on spontaneous metastasis therefore mirrored its effects on the primary tumours, i.e. a degree of tumour immunogenicity and immunocompetent host seemed to be obligatory for successful inhibition of tumour growth in both cases. It is important to note that in our studies the animals remained healthy throughout and beyond their 3–4 week course of retinoid treatment. Also, although the effects on a local tumour and its metastases were comparable, the reduction in secondary disease was not merely a consequence of limited primary tumour growth since tumours in which retinoids induced a growth delay were allowed to remain *in situ* until they achieved the same size as control tumours at excision (i.e. 8–10 mm).

Can retinoids significantly influence tumours of weak or undetectable immunogenicity?

The data presented so far suggest that retinoids can have profound effects on the primary and secondary growth of tumours which evoke a strong immune response in their hosts (directed either against H-2 antigens, virally associated antigens, or those induced by mutagenic carcinogens). However, there is no doubt that such strong immunogens, while useful for exploring the limits of immunotherapeutic and immunoadjuvant protocols, and for dissecting which components of the host defence mechanism can be stimulated to eliminate antigenic neoplastic cells, are nevertheless of little relevance to cancer in man. Monoclonal antibodies have identified many human tumour-associated "antigens" [44]. These are by and large normal cellular components abnormally expressed, or differentiation antigens not normally present on adult tissue or non-proliferating cells. Whether any such antigens can act (or be induced to act) as immunogens or whether all are immunologically inert in the host due to self-tolerance remains to be determined.

There is some evidence that retinoids, alone or in combination with other treatments, can influence weakly or non-immunogenic tumour systems under defined conditions. Malkovsky *et al.* [30, 31] studied the effects of diet supplemented with Vitamin A acetate (RAA) on the growth of a methylcholanthrene-induced sarcoma (McSa-1) and a Harvey sarcoma virus induced tumour (HT 3-2.1) in syngeneic mice. The growth of the immunogenic McSa-1 was inhibited by RAA, but neither the RAA diet nor pre-immunisation could increase the resistance of mice to the HT3-2.1 tumour, which was therefore considered non-immunogenic. However, a combination of pre-immunisation and the RAA-supplemented diet specifically increased the survival of mice challenged with HT3-2.1 cells, allowing (or inducing) their "hidden" immunogenicity to be expressed. The maximum resistance to tumours was observed when dietary supplements were initiated 3–4 weeks before tumour challenge; no effects were obtained if treatment was delayed until the day of tumour inoculation. Similar results were reported by Patek *et al.* [22] with the weakly immunogenic EL4 lymphoma, and we also found that the effects of retinoids on tumour growth and metastasis were dose and time dependent. Thus, tumours expressing strong antigens (e.g. FS6) could be controlled by relatively low doses of retinoid administered after tumour cell inoculation, whereas tumours of weak or undetectable immunogenicity (e.g. FS6M1) required higher doses and/or pretreatment for an effect to be obtained (see Fig. 2 and Table 2; and S. A. Eccles *et al.**). Even with this "optimal" regime, however, certain tumours were still refractory to retinoid treatment *in vivo*.

Several investigators have shown that, in systems where retinoic acid analogues alone produced little or no inhibition of tumour development, their combination with other treatments produced additive or synergistic benefit. Most of the studies used retinol or retinyl palmitate, and enhancement of the effects of radiation [43, 45], cytotoxic drugs [14, 45–48] and bacterial adjuvants (e.g. BCG and *Corynebacterium parvum*) [15, 16, 21] were obtained. Tannock *et al.* [43] reported that Vitamin A could reduce the dose of X-irradiation necessary to control a mouse sarcoma, and that this effect was abolished by prior immunosuppression. Seifter *et al.* [45] found that Vitamin A or β-carotene (a dietary precursor of Vitamin A) combined with local irradiation caused 100% "complete" regression of a murine adenocarcinoma, whereas mice receiving radiation or dietary supplements alone all died within 3 months. The remarkable feature of this series of experiments is that, if the diet was returned to normal *one year* after the tumours had disappeared, most of the mice that had received supplementary Vitamin A, and about 30% of those fed β-carotene developed recurrent tumours; all of the mice still maintained on either diet remained tumour free. The greater residual protection afforded by β-carotene was suggested as being due to its greater tissue storage. Nathanson *et al.* [14] showed that Vitamin A could enhance the effect of low dose cyclophosphamide against an osteogenic sarcoma and mammary carcinoma in mice, but that pretreatment with retinoids was necessary to obtain real therapeutic advantage.

The superiority of pretreatment regimens [14, 22, 23, 35, 40, 41*] plus the facts that (a) in some systems inhibition of tumour growth may take about 10 days to become evident [48,*] (b) the synergistic

* S. A. Eccles *et al.*, *Cancer Immunol. Immunother.*, in press (1985).

effects of retinoids can be abolished by immunosuppression [43], and (c) tumour cells are not necessarily destroyed, but may merely be held in check [45], argue against a direct cytotoxic effect of retinoids augmenting the cytotoxic effects of radio- or chemotherapy. Rather, these features suggest that in combined therapy their effects are again primarily mediated by immunopotentiation of host anti-tumour effect mechanisms.

Mechanism of action of retinoids in inhibitory effects on tumour growth in vivo

Aduvjant properties. Dresser [49] defined as an adjuvant any agent capable of aborting the tolerance normally evoked by a soluble antigen, bovine gamma globulin (BGG), and showed that immunity to BGG could be induced if mice were pretreated with Vitamin A [50]. Since then many retinoids have been shown to possess true adjuvant activity in the induction of humoral and/or cell-mediated immunity to a variety of antigens [9, 24, 51–54].

Of more direct relevance to the present discussion is the evidence that a variety of retinoids can augment the host response to weak transplantation- and tumour-associated antigens. RAA and retinol were shown to hasten H-Y incompatible (male→female) skin graft rejection in syngeneic mice, and these compounds and retinyl palmitate specifically increased the resistance of mice pre-immunised with syngeneic weakly immunogenic tumours [29–31, 55]. Also, lymphoid cells from retinoid-treated mice have been shown to possess an enhanced ability to kill tumour cells *in vitro* [30, 56, 57] and *in vivo* [58, 59] and to transfer immunity to naive recipients [55, 60]. It is of interest that, whereas low doses (25–300 μg/mouse/day) of β-RA and all doses tested of 13-*cis* RA, retinyl palmitate and TMMP stimulated immunity [56, 58, 59] high doses of β-RA were toxic and immunosuppressive. This fact may help to explain the generally more reproducible and beneficial effects of low dose RA and retinol [27, 61] and of the less toxic aromatic analogues [6–8, 11].

The enhanced immunogenicity of tumours in retinoid-treated hosts could be due to an effect on the expression of tumour antigens and/or the response of the host to existing antigens. Regarding the former, as noted above, soluble antigens (which many tumours are known to shed) may be rendered immunogenic rather than tolerogenic by retinoids, and the effects of retinoids on tumour cell or stromal cell membranes may modify antigen expression. This effect is apparently demonstrated by an allogeneic transplantable rat chondrosarcoma which regresses in rats treated with 13-*cis* RA [62] TMMP, TMMP-EE and TMMP-EA [63]. It has been suggested that specific inhibition of glycosaminoglycan synthesis in neoplastic cartilage by retinoids results in loss of matrix and exposure of antigens which renders the tumour susceptible to immunological rejection [63–65]. In other systems it was clearly shown that the antigenicity of tumour cells was unaffected by retinoid treatment *in vitro* or *in vivo* [30, 56].

It has been proposed [66] that a common mechanism of action of adjuvants is to alter lymphocyte circulation and increase their localisation in stimulated lymph nodes, one of the most important events in the initiation of an immune response [67]. Dresser *et al.* [68] showed that retinol is indeed able to mediate this effect, and inhibition of trapping of labelled lymphocytes in antigen-stimulated nodes of Vitamin A deficient rats has been described recently [69].

Although the component parts of the immunological network exist in a complex and interdependent array [70, 71], it is important to try to dissect further the site(s) of action of retinoids *in vivo*.

T lymphocytes. The preceding sections have indicated that retinoids can specifically augment antigen-induced T lymphocyte proliferation and activation. Also, the fact that T cell selective immunodeprivation (e.g. invoked by athymy or Cyclosporin A) can abrogate the retinoid-induced inhibition of tumour growth and/or metastasis suggests a pivotal role for this cell type in the *in vivo* response. More detailed investigations have revealed that it is the Ly 1 subset of the mouse T-lymphocyte population that is increasingly represented in lymphoid tissues responding to retinoids, and that it is responsible for transfer of resistance to unprimed hosts [31, 60]. This is a significant observation, since although cytotoxic lymphocytes of Ly 23 phenotype [72] capable of killing tumour cells *in vitro* are induced by RA [57] these cells have little or no effects *in vivo* [73]. Rather, increasing evidence suggests that the non-cytotoxic T-"helper" (Ly 1) lymphocyte population is responsible for inhibition of syngeneic tumour growth [73–77] by eliciting a delayed hypersensitivity reaction involving other mononuclear cells. Also, since the induction of the activity of Ly 23 and Ly 1 cells is macrophage dependent, the observed effects do not prove that either of these T cell phenotypes, or their Ly 123 precursors constitute the prime target for retinoid immunopotentiation *in vivo*.

Mononuclear phagocytes. Although the T-cell dependence of certain retinoid effects *in vivo* is readily demonstrated in T-cell deficient animals, the equivalent state of monocyte and macrophage neutralisation is not easily (if at all) achievable. Dendritic antigen-presenting cells and/or macrophages are critically important at each stage in the evolution of immune responses to tissue antigens, and the latter have also been directly implicated in an effector capacity in both non-specific and specific elimination of neoplastic cells [36, 76]. Their activities, in turn, are dependent on, or enhanced by, lymphokines [77] and some may be indirectly abrogated by T-cell depletion.

We found that silica and carrageenan (which are toxic to phagocytic cells [78–81]) did not interfere with the inhibitory effects of TMMP-EE on local tumour growth, but did prevent the abolition of DM6 carcinoma metastasis by retinoid.* These agents have also been shown to potentiate local tumour growth [82, 83] or metastasis [84], and the complexity of events elicited after MPS damage [80] and the divergent effects on primary tumours and metastases in different systems render interpretation of the combined effects with retinoid difficult. Further studies

* S. A. Eccles *et al.*, *Cancer Immunol. Immunother.*, in press (1985).

with a wider variety of macrophage inhibitors, retinoids and tumours are clearly required. There is, however, already some evidence that retinoids can influence macrophage activity. Frost and Lance [66] suggested a link between macrophage function and the action of adjuvants including retinoids, and the stimulation of antibody production to the T-independent antigen dinitrophenylated-lysyl-Ficoll was interpreted as being due to stimulation of the MPS and enhanced entrapment of lymphocytes in the spleen [85]. Rhodes and Oliver [86] showed that physiological concentrations of retinol could exert potent regulatory effects on macrophage function, reducing Fc receptor expression and increasing production of a tumoricidal enzyme, arginase [87]. Similarly, Tachibana *et al.* [88] showed that RA, retinol and retinyl palmitate could render rat alveolar macrophages tumoricidal against syngeneic mammary adenocarcinoma cells. Direct interaction with retinoid *in vitro* was also able to mediate this effect.

It is possible, therefore, that indirect or direct activation of macrophages by retinoids may contribute to inhibition of tumour growth *in vivo*. The fact that xenografted tumours in athymic animals may be susceptible to growth inhibitory effects by retinoids [61] is sometimes quoted as evidence of direct (non-immunological) intervention. However, while T-cell dependent immune functions are deficient in such animals, non-specific defence mechanisms mediated by macrophages and NK cells are not [89, 90]. Indeed, these effectors may be more important than T-cells in the response to xenoantigens [91, 92] and their cytotoxic activity can be directly stimulated by adjuvants [93]. Retinoid treatment of neonatal nu/nu animals whose NK and macrophage functions have not matured would clarify whether tumours could respond directly to retinoids *in vivo*.

In studies in our laboratories, the level of tumour-infiltrating host cells (mainly mononuclear phagocytes) was increased in TMMP-EE-treated animals, suggesting an enhanced local cell-mediated immune response to tumour antigens which may secondarily limit dissemination and metastasis [36, 94–97]. Since Mori and Kobayashi [98] also noted that TMMP-EE induced marked histiocytic and "round cell" infiltration in primary chemically-induced tumours, it is unlikely that this phenomenon is an artefact of transplanted tumours.

NK cells. The role of NK (natural killer) cells in the growth and dissemination of solid tumours *in vivo* remains controversial since most established non-lymphoid tumour cells need to be cultured *in vitro* for some time before any sensitivity to NK cell activity is acquired. All of the tumours in our studies, and those of Medawar and colleagues are resistant to NK cells, and yet responded to retinoid treatment *in vivo*. Some studies have shown that retinoids can activate NK cells [99, 100], others that their effects are inhibitory [101]. Thus, there is no consistent association between retinoid response to tumours and stimulation of NK activity *in vivo*.

Endothelial cells. A much neglected yet interesting and important tissue is the vascular endothelium, the embryonic development of which is retinoid dependent [102]. In the adult animal, far from being an inert conduit, it is clear that there are dynamic interactions between immunocompetent cells, tumour cells and endothelial cells which may be significant in the initiation, progression and resolution of immune responses [102], and tumour growth and metastasis. Cells of the high endothelial venules (HEV) in stimulated lymph nodes produced a sulfated glycolipid that may interact with recognition molecules on lymphocytes and contribute to their accumulation [103, 104], and endothelial cell proliferation is stimulated by factors produced by T-helper cell and macrophage interactions [105, 106]. Activated lymphocytes can also induce expression of HLA-DR (Ia like) antigens on endothelial cells [107]. These determinants can aid the presentation of antigens, and their co-expression with tumour antigens may influence the developing immune response. In addition, growing tumours evoke neovascularisation and must interact with capillary endothelial cells in their invasion of surrounding normal tissue and, again, following dissemination in blood and lymph, extravasation must occur before metastatic colonisation of secondary sites can be accomplished.

There are many potential ways in which retinoids may influence these interactions, e.g. by their activation of T-helper cells and macrophages, and by their role in the synthesis of glycoconjugates such as glycosaminoglycans, proteoglycans, glycoproteins and glycolipids which are essential to specific cell–cell recognition, adhesion and interaction [9, 69, 108, 109]. With techniques currently available (e.g. the cultivation of capillary endothelial cells and their coculture with tumour cells and/or immunocompetent cells, and the quantitation of lymphocyte traffic through isolated perfused nodes), investigations of retinoid activity in these systems could yield important new information on their biological and biochemical effects.

Prostaglandins. The prostaglandins represent a major class of intercellular messengers, and may modulate a variety of immunological responses [110] and also the metastatic process [111]. Retinoids have been shown to induce the synthesis of prostaglandins [112], and thus may indirectly affect blood flow, lymphocyte migration, chemotaxis, platelet activity, cAMP-dependent functions and a variety of interactions between normal and malignant cells.

Retinoids and cell membranes

Space does not permit more than a brief summary of the interactions of retinoids with normal and neoplastic cell membranes, which have been the subject of several reviews [4, 9, 10]. The present discussion will centre only on those aspects which may or may not determine the *in vivo* activity of retinoids.

Cellular retinol and retinoid acid binding proteins (cRBP and cRABP). Cellular binding proteins for retinol and RA have been demonstrated on a variety of cells, and this has led to the suggestion that their modes of action resemble steroid hormones; i.e. that retinoid–protein conjugates are transported to the cell nucleus where they bind to chromatin and affect gene expression [113, 114]. However, *in vivo* this action should be independent of host immune status (whereas many tumour growth inhibitory effects apparently are not). Also, there is no clear-cut associ-

ation between the levels of these proteins and the susceptibilities of different tumours to retinoid effects, and indeed in several cases profound effects have been obtained in the absence of either protein [115–118]. These data suggest that the tumour-inhibitory effects of retinoids can be dissociated from their differentiation-promoting activity in many cases.

Glyconconjugate biosynthesis. In vitro and *in vivo* investigations have revealed that retinol can control glycosylation of specific glycoproteins, and that retinoic acid (and other retinoids) may substitute for Vitamin A in promoting glycoprotein biosynthesis [108]. It is not yet known whether retinoid regulation is exerted at the level of transcription, or at a post-transcriptional stage by direct effects on lysosomal or cell surface membranes [119]. It has already been noted that glycoconjugates are critical in the social behaviour of cells, and changes in carbohydrate expression and glycosyltransferases have been proposed to account for the acquisition of enhanced metastatic capacity during tumour progression, and may explain some of the "organ-selective" patterns of metastasis [34, 120]. Recent evidence suggests a further role for glycosyltransferases in T-cell recognition and possibly other more primitive recognition systems. It has been proposed that the major histocompatibility (MHC) locus controls families of carbohydrate, as well as protein histocompatibility antigens, and that the glycosyltransferases which construct the former antigens therefore play a central role in MHC restricted recognition by T-cells and act as "anti-self" receptors [121]. The implications for retinoid modulation of this system by interactions at the gene level or at the cell surface are obvious.

Fibronectin (FN) is a cell surface glycoprotein, widely distributed on cells and in sera, and is a major structural component of connective tissue, in particular the basal lamina of endothelial cells [122, 123]. It has been suggested that the capacity of tumour (and embryonic) cells for migration and invasion is related to their reduced levels of cell bound FN compared with normal adult cells [124]. Retinoids have been shown to be capable of stimulating the production of FN in fibroblasts and papilloma cells, but not in transformed fibroblasts or carcinoma cells [125, 126]. Thus, while this activity of retinoids may play a role in the inhibition of tumour promotion in carcinogenesis, it is unlikely to account for the inhibition of growth and metastasis of frankly malignant cells.

Lysosomal membrane labilisation. Retinoids have been shown to labilise cell membranes and cause release of lysosomal enzymes with consequent cell lysis [127, 128]. This activity has been proposed to account for their ability to promote the anti-tumour effects of radiation [43] and chemotherapeutic drugs [51]. However, RAA was found to be 1/100th, and RA 1/10th as "membrane-active" as retinol [47], yet all three compounds have significant effects on tumours *in vivo* (first and second sections of this paper). Also, the doses of retinol used by Rettura, Seifter and colleagues [25, 27, 45] were much lower than those required to cause lysosomal damage, and yet gave significant inhibition of tumour growth alone, or in combination with X-irradiation. It is likely rather that the toxic effects of retinoids, and some of the symptoms of hypervitaminosis A are due to the release of lysosomal hydrolases. Therapeutic effects are probably not dependent upon this activity except perhaps in that the release of arginase and other enzymes from retinoid-stimulated macrophages may contribute to tumour cell destruction.

Conclusions, outlook and future work

The direct effects of retinoids on the growth and differentiation of normal and neoplastic cells are rightly receiving much attention, and it was an interesting exercise to enquire instead into the role of indirect, host-mediated activities of retinoids in the control of established tumour growth and metastasis, some of which are illustrated in Fig. 3. It came as some surprise to discover the extent to which the apparently confusing data could be resolved when tumour immunogenicity and host immunocompetence were taken into account, particularly if a liberal interpretation of "immunity" to include non-specific defences is adopted. While by no means providing a complete alternative explanation of *in vivo* retinoid action, I think the data sufficiently compelling to merit the inclusion of assays of immunopotentiatory activity in the evaluation of new retinoids. There is some evidence that different classes of antigens (e.g. protein vs carbohydrate) may evoke qualitatively and quantitatively different immune responses; also that early incipient neoplasia, progressively growing local tumours, and disseminated micrometastases can invoke (or subvert) different components of the host immunoregulatory network. Further work on the structural requirements of retinoids (and their metabolites) for optimum and selective effects on proliferation, differentiation and potentiation of all the different facets of "immunity" is imperative. Also, analysis of the precise cellular and molecular bases for these physiological effects is required, and the ever-increasing sophistication of recombinant DNA technology is expected to shed new light on the dark and mysterious machinery of retinoid-induced changes in gene expression and membrane function.

The fact that pretreatment with retinoids and/or combination with other treatment modalities was generally found to be necessary to obtain any effects on tumours of very weak or undetectable immunogenicity suggests a limited potential for the current generation of retinoids in an adjuvant therapeutic setting. However, the possibility exists that this may be improved if means are found to render tumour associated antigens intrinsically more immunogenic in the autochthonous host, or if selective inhibition of suppressor cell activity could be achieved. In the meantime, retinoids are already being tested clinically, and promising results are being obtained in prevention and therapy of precancerous and cancerous conditions [129–131] and in the restoration of immunocompetence in patients suffering impairment of lymphocyte or macrophage function due to malignant disease, surgery or chemotherapy [132–134]. It is hoped that the combined approaches of analytical and empirical research will yield new insights into the complex and diverse activities of retinoids, and thus allow their full potential to be realised.

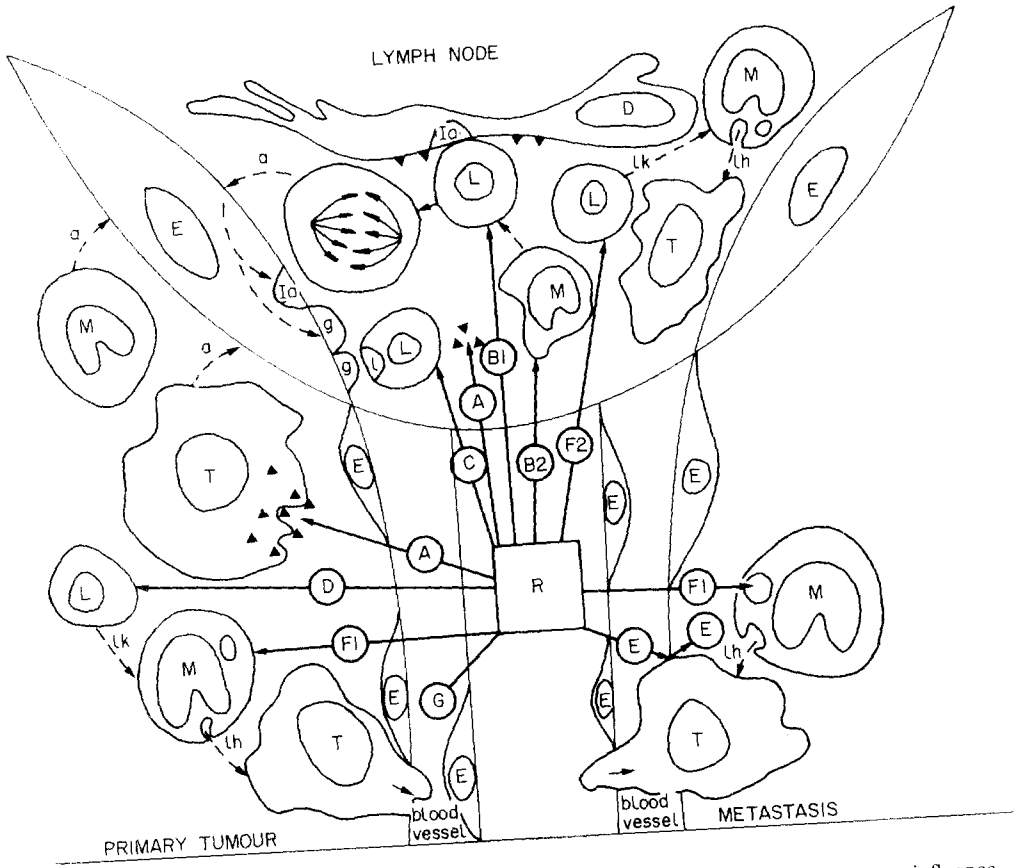

Fig. 3. Schematic representation of some direct and indirect effects of retinoids which may influence tumour growth and metastasis *in vivo*. (A) Immunogenicity of membrane-bound and shed soluble antigens of tumour cells (T) may be enhanced. (B) Activated T-killer (Ly23) or T-helper (Ly 1) lymphocytes (L) are induced directly (B1) or indirectly (B2) via activation of macrophages (M). (C) Trapping of L in lymph nodes is enhanced via lectin (l)-dependent glycoconjugate (g)-associated interaction with endothelial cells (E) and dendritic cells (D). Activated L, in turn, stimulate expression of g and Ia-like antigens on E which may further augment antigen presentation and L accumulation. E proliferation is induced by angiogenesis factors (a) produced by T, or activated M and L. (D) Enhanced accumulation of M in primary tumours (probably in response to lymphokines, lk) may limit T invasion and dissemination. (E) Glycosyltransferase activity and altered glycoprotein metabolism may influence localisation of metastases, and binding of T by M. (F) M activated directly by R (F1) or indirectly (F2) by lk in primary tumour or in target organs for metastasis (e.g. lymph node, lung) may kill T by release of lysosomal hydrolases (lh). (G) R can affect vascular permeability, blood flow, activity of L and M and platelets, prostaglandin levels, etc., and thus survival of circulating T.

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REFERENCES

1. S. B. Wolbach and P. R. Howe, *J. exp. Med.* **42**, 753 (1925).
2. Y. Fujimaki, *J. Cancer Res.* **10**, 469 (1926).
3. T. Kummet, Te Moon and F. L. Meyskens, *Nutr. Cancer* **5**, 96 (1983).
4. D. E. Ong and F. Chytil, *Vitams Horm.* **40**, 105 (1983).
5. G. H. Clamon, *Med. pediatr. Oncol.* **8**, 177 (1980).
6. W. Bollag and H. R. Hartmann, *Cancer Survs* **2**, 293 (1983).
7. W. Bollag and A. Matter, *Ann. N.Y. Acad. Sci.* **359**, 9 (1981).
8. H. Mayer, W. Bollag, R. Hanni and R. Ruegg, *Experientia* **34**, 1105 (1978).
9. R. Lotan, *Biochim. biophys. Acta* **605**, 33 (1980).
10. M. B. Sporn and A. B. Roberts, *Cancer Res.* **43**, 3034 (1983).
11. W. Bollag, *Chemotherapy* **21**, 236 (1975).
12. Y. Ito, *Eur. J. Cancer* **17**, 35 (1981).
13. D. P. Stewart, R. J. Speer, H. J. Ridgway and J. M. Hill, *J. clin. Haemat. Oncol.* **9**, 235 (1979).
14. L. Nathanson, C. L. Maddock and T. C. Hall, *J. clin. Pharmac.* **9**, 359 (1969).
15. T. Kurata and Mickshé, *Oncology* **34**, 212 (1977).
16. Z. P. Pavelic, S. Dave, S. Bialkowski, R. L. Priore and W. P. Greco, *Cancer Res.* **40**, 4617 (1980).
17. S. Baron, K. M. Kleyn, J. K. Russell and J. E. Blalock, *J. natn. Cancer Inst.* **67**, 95 (1981).
18. I. Cameron, B. Grubbs and W. Rogers, *Cancer Treat. Rep.* **63**, 477 (1979).
19. E. L. Felix, B. Loyd and M. H. Cohen, *Science* **189**, 886 (1975).

20. E. L. Felix, M. H. Cohen and B. C. Loyd, *J. surg. Res.* **21**, 307 (1976).
21. M. S. Meltzer and B. E. Cohen, *J. natn. Cancer Inst.* **53**, 585 (1974).
22. P. Ø. Patek, J. L. Collins, G. Yogeeswaran and G. Dennert, *Int. J. Cancer* **24**, 624 (1979).
23. P. B. Medawar and R. Hunt, *Immunology* **42**, 439 (1981).
24. T. J. Athanassiades, *J. natn. Cancer Inst.* **67**, 1153 (1981).
25. G. Rettura, A. Schittek, M. Hardy, S. M. Levenson, A. Dimetriou and E. Seifter, *J. natn. Cancer Inst.* **54**, 1489 (1975).
26. E. Seifter, G. Rettura and S. M. Levenson, *J. natn. Cancer Inst.* **67**, 467 (1981).
27. G. Rettura, F. Stratford, S. M. Levenson and E. Seifter, *J. natn. Cancer Inst.* **69**, 73 (1982).
28. J. M. Deneufbourg, *Biomedicine* **31**, 122 (1979).
29. P. H. Chauvenet and R. E. Paque, *Int. J. Cancer* **30**, 187 (1982).
30. M. Malkovsky, C. Doré, R. Hunt, L. Palmer, P. Chandler and P. B. Medawar, *Proc. natn. Acad. Sci. U.S.A.* **80**, 6322 (1983).
31. M. Malkovsky and P. B. Medawar, *Immun. Today* **5**, 178 (1984).
32. R. Lotan and G. L. Nicolson, *J. natn. Cancer Inst.* **59**, 1717 (1977).
33. S. A. Eccles, *J. Path.* **141**, 333 (1983).
34. S. C. Barnett and S. A. Eccles, *Clin. expl Metastasis* **2**, 15 (1984).
35. S. A. Eccles, in *Tumor Progression and Markers* (Eds. K. Lapis and M. Price), p. 183. Kugler, Amsterdam (1982).
36. S. A. Eccles, in *Tumour Immunity and Prognosis: The Role of Mononuclear Cell Infiltration* (Ed. J. S. Haskill), p. 37. Marcel Dekker, New York (1982).
37. P. Alexander and S. A. Eccles, *Transplantn Proc.* **XVI**, 486 (1984).
38. P. Alexander and S. A. Eccles, in *Cancer Invasion and Metastasis: Biologic and Therapeutic Aspects* (Eds. G. L. Nicolson and L. Milas), p. 293. Raven Press, New York (1984).
39. L. Weiss and E. C. Holyoke, *J. natn. Cancer Inst.* **43**, 1045 (1969).
40. D. M. Morré, T. M. Kloppel, A. L. Rosenthal and P. C. Fink, *J. Nutr.* **110**, 1629 (1980).
41. D. J. Morré, K. E. Creek, D. M. Morré and C. L. Richardson, *Ann. N.Y. Acad. Sci.* **359**, 367 (1981).
42. A. Tannenbaum and H. Silverstone, *Cancer Res.* **13**, 532 (1953).
43. I. F. Tannock, H. D. Suit and N. Marshall, *J. natn. Cancer Inst.* **48**, 731 (1972).
44. K. E. Hellström, I. Hellström and J. P. Brown, *Springer Semin. Immunopath.* **5**, 127 (1982).
45. E. Seifter, G. Rettura, J. Padawer, F. Stratford, P. Goodwin and S. M. Levenson, *J. natn. Cancer Inst.* **71**, 409 (1983).
46. M. H. Cohen, *J. natn. Cancer Inst.* **48**, 927 (1972).
47. M. H. Cohen and P. P. Carbone, *J. natn. Cancer Inst.* **48**, 921 (1972).
48. Y. Tomita, K. Himeno, K. Nomoto, H. Endo and T. Hirohata, *J. natn. Cancer Inst.* **68**, 823 (1982).
49. D. W. Dresser, *Clin. expl Immun.* **3**, 877 (1968).
50. D. W. Dresser, *Nature, Lond.* **217**, 527 (1968).
51. B. E. Cohen and I. K. Cohen, *J. Immun.* **111**, 1376 (1973).
52. M. Jurin and I. F. Tannock, *Immunology* **23**, 283 (1972).
53. J. K. Spitznagel and A. C. Allison, *J. Immun.* **104**, 119 (1970).
54. A. C. Allison and A. J. S. Davies, *Nature, Lond.* **233**, 330 (1971).
55. Y. Tomita, *Nutr. Cancer* **5**, 187 (1983).
56. G. Dennert and R. Lotan, *Eur. J. Immun.* **8**, 23 (1978).
57. G. Dennert, C. Crowley, J. Kouba and R. Lotan, *J. natn. Cancer Inst.* **62**, 89 (1979).
58. R. Lotan and G. Dennert, *Cancer Res.* **39**, 55 (1979).
59. M. Glaser and R. Lotan, *Cell. Immun.* **45**, 175 (1979).
60. M. Malkovsky, A. J. Edwards, R. Hunt, L. Palmer and P. B. Medawar, *Nature, Lond.* **302**, 338 (1983).
61. N. T. Wetherall, W. M. Mitchell and S. A. Halter, *Cancer Res.* **44**, 2393 (1984).
62. C. Heilmann and R. L. Swarm, *Fedn Proc.* **35**, 822 (1975).
63. P. W. Trown, M. J. Buck and R. Hansen, *Cancer Treat. Rep.* **60**, 1647 (1976).
64. S. S. Shapiro, M. Bishop, J. P. Poon and P. W. Trown, *Cancer Res.* **36**, 3702 (1976).
65. F. Langer and A. E. Gross, *J. Bone Jt Surg.* **56**, 297 (1974).
66. P. Frost and E. M. Lance, *Immunology* **35**, 63 (1978).
67. J. Sprent, J. F. A. P. Miller and G. F. Mitchell, *Cell. Immun.* **2**, 171 (1971).
68. D. W. Dresser, R. N. Taub and A. R. Krantz, *Immunology* **18**, 663 (1970).
69. H. Takagi and K. Nakano, *Immunology* **48**, 123 (1983).
70. E. R. Unanue, *New Engl J. Med.* **303**, 977 (1980).
71. E. J. Wing and J. S. Remington, *Cell Immun.* **30**, 108 (1977).
72. H. Cantor and E. A. Boyse, *Cold Spring Harb. Symp. Quant. Biol.* **41**, 23 (1976).
73. A. K. Bhan, L. L. Perry, H. Cantor, R. T. McCluskey, B. Benacerraf and M. I. Greene, *Am. J. Path.* **102**, 20 (1981).
74. G. Forni and M. Giovarelli, *J. Immun.* **132**, 527 (1984).
75. P. D. Greenberg, M. A. Cheever and A. Fefer, *J. exp. Med.* **154**, 952 (1981).
76. I. J. Fidler and G. Poste, *Springer Semin. Immunopath.* **5**, 161 (1982).
77. I. J. Fidler and A. Raz, in *Lymphokines* (Ed. E. Pick), Vol. 3, p. 345. Academic Press, New York (1981).
78. A. C. Allison, J. S. Harington and M. Birbeck, *J. exp. Med.* **124**, 141 (1966).
79. N. Pearsall and R. Weisser, *J. reticuloendothal. Soc.* **5**, 107 (1968).
80. M. Levy and E. F. Wheelock, *J. Immun.* **115**, 41 (1975).
81. P. J. Catanzaro, H. J. Schwartz and R. Graham, *Am. J. Path.* **64**, 387 (1971).
82. E. Lotzová and E. R. Richie, *J. natn. Cancer Inst.* **58**, 1171 (1977).
83. R. Keller, *J. natn. Cancer Inst.* **57**, 1355 (1976).
84. A. Mantovani, R. Giavazzi, N. Poletarutti, F. Spreafico and G. Garattini, *Int. J. Cancer* **25**, 617 (1980).
85. K. Sugimura, I. Azuma, Y. Yamamura, I. Imada and H. Morimoto, *Int. J. Vit. nutr. Res.* **46**, 464 (1976).
86. J. Rhodes and S. Oliver, *Immunology* **40**, 467 (1980).
87. D. O. Adams and C. F. Nathan, *Immun. Today* **4**, 166 (1983).
88. K. Tachibana, S. Sone, E. Tsubura and Y. Kishino, *Br. J. Cancer* **49**, 343 (1984).
89. R. B. Herberman, in *The Nude Mouse in Experimental and Clinical Research* (Eds. J. Fogh and B. C. Giovanella), p. 135. Academic Press, New York (1978).
90. C. Cheers and R. Waller, *J. Immun.* **115**, 844 (1975).
91. G. Dennert and E. S. Lennox, *J. Immun.* **111**, 1844 (1973).
92. S. A. Eccles, in *Immunodeficient Animals for Cancer Research* (Ed. S. Sparrow), p. 167. Macmillan Press, London (1980).
93. J. D. Gray and C. G. Brooks, *Cell Immun.* **53**, 405 (1980).

94. S. A. Eccles and P. Alexander, *Nature, Lond.* **250**, 667 (1974).
95. S. A. Eccles and P. Alexander, *Nature, Lond.* **257**, 52 (1975).
96. S. A. Eccles, J. M. Styles, S. M. Hobbs and C. J. Dean, *Br. J. Cancer* **40**, 802 (1979).
97. S. A. Eccles, S. E. Heckford and P. Alexander, *Br. J. Cancer* **42**, 252 (1980).
98. M. Mori and K. Kobayashi, *Cell. molec. Biol.* **27**, 27 (1981).
99. R. H. Goldfarb and R. B. Herberman, *J. Immun.* **126**, 2129 (1981).
100. R. B. Herberman and J. R. Ortaldo, *Science* **214**, 24 (1981).
101. J. Abb, H. Abb and F. Deinhardt, *Int. J. Cancer* **30**, 307 (1982).
102. W. M. Baldwin, *Immun. Today* **3**, 267 (1982).
103. M. T. Drayson, M. E. Smith and W. L. Ford, *Immunology* **44**, 125 (1981).
104. Y. H. Chin, G. D. Carey and J. J. Woodruff, *J. Immun.* **125**, 1764 (1980).
105. Y. A. Sidky and R. Auerbach, *J. exp. Med.* **141**, 1084 (1975).
106. P. J. Polverini, R. S. Cotran, M. A. Gimbrone and E. R. Unanue, *Nature, Lond.* **269**, 804 (1977).
107. P. Stastny and G. Nunez, in *Transplantation and Clinical Immunology* (Eds. J. L. Touraine, J. Traeger, H. Betvél, J. Brochier, J. M. Dubernard, J. P. Revillard and R. Trian). Vol. XIII, p. 132. Excerpta Medica, Amsterdam (1981).
108. L. M. de Luca, *Vitams Horm.* **35**, 1 (1977).
109. I. A. King and A. Tabiowa, in *Retinoids, Advances in Basic Research and Therapy* (Eds. C. E. Orfanos, O. Braun-Falco, E. Farber, C. Grupper, H. K. Polano and R. Schluppi), p. 473. Springer, Berlin (1981).
110. J. S. Goodwin and D. R. Webb, *Clin. Immun. Immunopath.* **15**, 106 (1980).
111. K. V. Honn, W. D. Busse and B. F. Sloane, *Biochem. Pharmac.* **32**, 1 (1983).
112. L. Levine and K. Ohuchi, *Nature, Lond.* **276**, 274 (1978).
113. F. Chytil and D. E. Ong, in *Receptors and Hormone Action* (Eds. B. W. O'Malley and L. Birnbaumer), p. 573. Academic Press, New York (1978).
114. F. Chytil and D. E. Ong, *Vitams Horm.* **36**, 1 (1978).
115. R. Lotan, D. E. Ong and F. Chytil, *J. natn. Cancer Inst.* **64**, 1259 (1980).
116. B. Sani and B. C. Titus, *Cancer Res.* **37**, 4031 (1977).
117. D. Douer and H. P. Koeffler, *J. clin. Invest.* **69**, 277 (1982).
118. P. R. Libby and J. S. Bertram, *Carcinogenesis* **3**, 481 (1982).
119. S. S. Shapiro and J. P. Poon, *Connect. Tissue Res.* **6**, 101 (1978).
120. V. Schirrmacher, P. Altevogt, M. Fogel, J. Dennis, C. A. Waller, D. Barz, R. Schwartz, R. Chieng-song-Popov, G. Springer, P. J. Robinson, T. Nebe, I. Vlodavsky, N. Paweletz, H. P. Zimmermann and G. Uhlenbruck, *Inv. Metastasis* **2**, 313 (1982).
121. C. R. Parish, H. C. O'Neill and J. T. Higgins, *Immun. Today* **2**, 98 (1981).
122. E. Ruoslahti, *Cancer Metastasis Rev.* **3**, 43 (1984).
123. K. M. Yamada and K. Olden, *Nature, Lond.* **275**, 179 (1978).
124. R. O. Hynes, A. T. Destree, M. E. Perkins and D. D. Wagner, *J. supramolec. Struct.* **11**, 95 (1979).
125. A. M. Jetten, M. E. R. Jetten, S. S. Shapiro and J. P. Poon, *Expl Cell Res.* **119**, 289 (1979).
126. S. D. Bolmer and G. Wolf, *Cancer Res.* **42**, 4465 (1982).
127. D. Brandes and E. Anton, *Lab. Invest.* **15**, 987 (1966).
128. J. T. Dingle and J. A. Lucy, *Biol. Rev.* **40**, 422 (1965).
129. S. Komiyama, I. Hirohita, S. Ryu, T. Nakashima and H. Endo, *Oncology* **35**, 253 (1978).
130. M. S. Soloway, *Urology* **23**, 88 (1984).
131. W. Bollag, *Lancet* **1**, 860 (1983).
132. J. Lundy, E. J. Lovett, S. M. Wolinsky and P. Conran, *Cancer, N.Y.* **43**, 945 (1979).
133. B. E. Cohen, G. Gill, P. R. Cullen and P. J. Morris, *Surgery Gynec. Obstet.* **149**, 658 (1979).
134. J. Rhodes, P. Stokes and P. Abrams, *Cancer Immun. Immunother.* **16**, 189 (1984).