Anti-inflammation induced by counter-irritation or by treatment with non-steroidal agents inhibits the growth of a tumour of non-detected immunogenicity

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Summary
Counter-irritation (CI) triggered by different non-specific irritant stimuli delayed the growth of a murine tumour of non-detected immunogenicity. The syngeneic LB tumour transplant by itself also induced CI and decreased the number of leukocytes migrating to a secondary s.c. irritant stimulus, e.g. sponge or carrageenan. On the other hand, partial inhibition of cell migration by treatment with either 0.5 mg kg\(^{-1}\) indomethacin or 6.3 mg kg\(^{-1}\) piroxicam retarded LB tumour growth, presumably by a mechanism unrelated to inhibition of immune responses by PGE\(_2\). It is suggested that CI may play a role in the early stages of concomitant resistance.

Activated macrophages (M\(_d\)) can display cytotoxicity against tumour cells and are considered an important host defence mechanism against neoplasia. However, M\(_d\)s may play a dual role in the growth of solid angiogenesis-dependent tumours (Folkman, 1985) since their presence at the site of incipient neoplastic growth is essential for neo-vascularisation. Although the mechanism involved remains obscure, a regulatory effect of inflammation on different stages of tumour growth has been proposed (Normann et al., 1988). In support of this hypothesis, Prehn & Prehn (1987) suggested that immunogenic neoplastic cells may benefit from interaction with inflammatory M\(_d\)s. Furthermore, it has been reported that treatment with non-steroidal anti-inflammatory agents (NSAIA) may restrict the growth of tumours of varying degrees of immunogenicity, probably by inhibiting prostaglandin E\(_2\) synthesis (Goodwin & Ceupens, 1983). Physiological anti-inflammation is mediated by complex and not completely understood mechanisms. One of these, CI, was defined as the ability of local irritation to exert an anti-inflammatory effect at a remote site in the body (Atkinson & Hicks, 1975). CI is non-specific and, as recently suggested, may inhibit the growth of immunogenic tumours (Normann et al., 1985b, 1987). This study was aimed at investigating the effect of CI and the administration of NSAIA on the growth of a solid tumour on non-detected immunogenicity.

Materials and methods

Animals
Male and female 8-week-old F\(_1\) hybrids (Balb/c x DBA/2) were raised in our animal house under standard conditions and fed ad libitum with Cargill (Buenos Aires) pelleted food and acidified tap water (final concentration, 50 mM HCl, pH = 2.8).

Tumour
The T-helper cell leukaemia (LB), which arose spontaneously in a 6-month-old Balb/c male mouse (Ruggiero et al., 1985) was, at the beginning of this study, in its 58th s.c. passage. When LB cells are injected s.c. a solid tumour develops in situ. Infiltration of lymph nodes, spleen and liver can be demonstrated at autopsy, but no solid metastases are detected. The LB cells were obtained from a solid tumour of a mouse inoculated s.c. 10 days before with 10\(^6\) cells. The tumour mass was removed, cut into small pieces and forced through a wire mesh. Cells were suspended in Hanks Balanced Salt Solution (HBSS) without calcium and magnesium (Flow Laboratories, McLean, VA, USA) to the appropriate density. Cell viability was assessed by trypan blue exclusion. Suspensions containing fresh cells were inoculated s.c. in the lateral flank and tumour growth was evaluated every other day by measuring tumour length and width with calipers. Tumour volume was determined using the formula proposed by Attia & Weiss (1966): \(V = 0.4 \times D \times d^2\) (\(V\), tumour volume in mm\(^3\), \(D\) and \(d\), longest and shortest diameters). The LB tumour exhibited no detectable immunogenicity in a rejection assay for Balb/c mice (Ruggiero et al., 1985). Previous work from our laboratory has shown that the number of animals bearing a tumour and survival were not modified by immunisation of F\(_1\) (Balb/c x DBA/2) s.c. with irradiated LB cells (data not shown).

Anti-inflammatory treatment
Indomethacin N-methyl-d-glucamine solution (IM 75, Montpellier, Buenos Aires) was diluted in 0.015M NaCl to obtain a dose of 0.5 mg kg\(^{-1}\) in 0.2 ml. Piroxicam (Pfizer, Argentina) was dissolved in dimethylsulphoxide, diluted 1 in 10 in 0.1M sodium bicarbonate, and further diluted in 0.015M NaCl to obtain the appropriate doses in 0.2 ml.

Induction and evaluation of counter-irritation
CI was triggered by implanting a nylon sponge (8–10 mm\(^3\)) s.c., or inoculating 10\(^6\) heat-killed Pseudomonas aeruginosa s.c. The effect of this treatment was compared with the CI produced by inoculating 10\(^6\) LB cells s.c. CI was evaluated by determining the inhibition of both oedema and cell migration induced by the inflammatory stimuli. Inhibition of oedema was evaluated 5 h after injecting 50 \(\mu\)l 1% carrageenan (Sigma Chemical Co., St Louis, MO, USA) into one of the hind-footpads. The degree of oedema was evaluated by weighing the amputated hind limbs. (Oyanagui, 1984). Cell migration was determined by s.c. implantation of a thoroughly washed, sterile polystyrene sponge either 24 h after administration of the irritant stimulus or at different times after tumour cell challenge. Twenty-four hours after implantation the sponge was removed, migrating cells were squeezed out and the total cell count was determined (Barrera et al., 1985). Inhibition of cell migration was also measured histopathologically in mice challenged s.c. with 10\(^6\) LB cells. At different times mice were injected intradermally (i.d.) with 20 pl of 1% carrageenan in the contralateral flank and killed 24 h later. Skin explants were fixed with 5% buffered formalin and tissue slices were stained with Haematoxylin and Eosin by standard procedures.
Table 1 Effect of piroxicam on mice survival after s.c. inoculation of LB tumour cells

| Days | Control surviving/total | Piroxicam surviving/total | P |
|------|-------------------------|---------------------------|---|
| 20   | 7/8                     | 8/8                       | 1 |
| 24   | 6/8                     | 8/8                       | 0.450 |
| 26   | 1/8                     | 8/8                       | 0.002 |
| 30   | 1/8                     | 7/8                       | 0.012 |
| 42   | 0/8                     | 2/8                       | 0.450 |

Statistics

Statistical analysis was performed using the Epistat package (T.L. Gustafson, Round Rock, TX, USA) in a personal computer. Data with normal distribution and homogeneous variances were compared by analysis of variance using the ANOVA program. Otherwise, data were analysed by the rank sum test using the Ranktest program.

Results

To determine whether CI affected tumour growth in vivo, a polystyrene sponge was implanted s.c. to generate local inflammation, followed 24 h later by 10⁵ tumour cells s.c. in the contralateral flank. Tumour growth was delayed (Figure 1) but tumour incidence and survival rates were not modified. Tumour growth was also retarded by anti-inflammatory treatment (Figure 2) and death of the mice (Table I) was delayed by 10 days. The tumour grew in all eight control mice while it didn’t grow in two out of eight piroxicam-treated mice. When treatment with piroxicam was continued beyond day 24 after tumour challenge, the solid tumour never developed although most animals died due to generalised infiltration by LB cells. In another experiment, 5-day-old tumours developing from a 10⁵ cell inoculum were excised, and the weight determined in untreated (3.38 ± 0.27 g) and piroxicam-treated (1.25 ± 0.09 g) mice. The number of cells recovered from tumours of control mice was 2.0 x 10⁸ while 1.3 x 10⁸ cells were recovered from tumours of treated mice. The smaller size of the tumours in treated mice, consequently, does not seem to be related to a decrease in tumoral oedema due to anti-inflammatory treatment. Moreover, incubation of the tumour mass at 60°C until constant weight revealed that the weight loss was 67% in untreated and 83% in treated mice, which indicates that the water relative content was higher in treated than in control mice.

The effects on inflammation of either CI triggered by different irritants or NSAIA treatment were evaluated by determining (i) the degree of oedema inhibition of the hindlimb footpads and (ii) the inhibition of cell migration towards the site where a second irritative stimulus was delivered. Carrageenan-induced paw oedema was inhibited by an s.c. implanted sponge (P = 0.032). A single dose of piroxicam 0.3 mg kg⁻¹ or indomethacin 0.5 mg kg⁻¹ also inhibited paw oedema (P = 0.0006 and P = 0.0002, respectively) (Figure 3). Cell migration towards a second s.c. sponge was diminished by treatment with piroxicam (P = 0.0043) or indomethacin (P = 0.0079). The presence of the first s.c. sponge as the primary irritant decreased the number of cells recovered from the contralateral, second s.c. sponge (P = 0.0086) (Figure 3). CI induced by an s.c. injection of 2 x 10⁶ formalin-killed gram-negative bacteria (Pseudomonas aeruginosa) also decreased the number of migrating cells from (1.5 ± 0.1) x 10⁶ (control mice) to 6.5 x 10⁵ cells ml⁻¹ (P = 0.0086).

The presence of LB tumour exerted anti-inflammatory activity as measured by decrease of paw oedema and cell

Figure 1 Modulation of tumour growth by counter-irritation induced by a sponge implanted s.c. 24 h before challenge with 10⁵ LB cells. Each point represents the median from six mice, (*) P<0.01 compared with controls.

Figure 2 Inhibition of tumour growth by treatment with piroxicam. Animals were treated i.p. with 0.3 mg kg⁻¹ piroxicam for 24 days, starting 72 h before challenge with 10⁵ LB cells. Each point represents the median from six mice, (*) P<0.01 when compared with controls.

Figure 3 Hind-paw oedema induced by carrageenan injection and cells migrating towards a secondary sponge placed s.c., in mice treated with 0.3 mg kg⁻¹ piroxicam, 0.5 mg kg⁻¹ indomethacin and mice with a primary s.c. sponge. Control mice were injected i.p. with saline and the group receiving a primary sponge were, in addition, sham-inoculated with a trocar. Since neither paw oedema nor cell migration were affected by this procedure, the results from a single control group are exhibited. Each bar represents the arithmetic mean ± s.e.m. from eight mice, (*) significant difference (see level of significance in Results).
migration. Paw oedema was diminished on day 4 or 6 after inoculation of $10^5$ LB cells ($P = 0.0069$) when compared with controls (Figure 4a). The number of cells recovered from the sponges were decreased by days 5 and 6 after tumour implantation ($P = 0.029$ and $P = 0.0004$, respectively). On day 7 a rebound effect was observed and the number of cells was increased ($P = 0.029$) (Figure 4b).

The effect of the tumour on cell migration towards an inflammatory i.d. stimulus (carrageenan) was confirmed histologically. In control mice, microscopy of skin explants revealed inflammation characterised by diffuse infiltration involving dermis and subcutaneous fatty tissue (Figure 5a). The infiltrate contained predominantly mononuclear cells (inset of Figure 5a). In contrast, mice bearing a solid LB tumour of 7 days growth exhibited inflammation in a band pattern restricted to a thin layer localised between dermis and fatty subcutaneous tissue (inset Figure 5b). When carrageenan was administered to mice bearing a 10-day tumour, severe inflammation developed, with intense infiltration localised in a band between dermis and fatty subcutaneous tissue, with only diffuse infiltration in these two areas (Figure 5c). At higher magnification, the density of the mononuclear cell infiltrate was even greater than that seen in untreated mice (inset Figure 5c vs. inset Figure 5a).

Discussion

There are two main aspects to be discussed in the light of the results from this study. One relates to CI as a natural mechanism of tumour growth inhibition, and the other is the therapeutic application of NSAIA to obtain inhibition of non-immunogenic tumour growth. Inhibition of inflammation by a secondary irritant stimulus due to CI has been demonstrated in diverse experimental systems. Supporting our results, there is previous evidence suggesting that inflammatory responses may be affected by the presence of a tumour. Snyderman et al. (1976) showed that experimental transplantation of a benzopyrene-induced sarcoma in rats prevented Mφ accumulation during inflammation. Furthermore, Normann et al. (1985a) have shown that transplantation of syngeneic tumour cells depressed Mφ inflammatory responses, probably through an increase in the endogenous levels of corticosterone (Normann et al., 1988). Our data demonstrate that, as with immunogenic tumours, a cancer of immunogenicity below our level of detection can also induce CI.

At least two important features of tumour-Mφ interaction should be considered. The first is that cellular immune responses occurring within a tumour can destroy it (Zbar et al., 1970). In fact, immunologically mediated tumour killing is frequently associated with an influx of Mφ to the site of neoplastic growth (Shin et al., 1975). On the other hand,
migratory cells, especially Mφ, are required during the early stages of solid tumour growth (Folkman & Cotran, 1976). There were two significant findings in our study: (i) inhibition of tumour growth by an inflammatory focus located away from the site of LB cell grafting; and (ii) inhibition of blood cell migration induced by the LB tumour towards the site where an irritant had been inoculated. Because the LB tumour itself can both induce CI and be affected by anti-inflammatory, it would be expected that a primary tumour inoculum could also inhibit the growth of a secondary inoculum of the same tumour.

Inhibition of experimental metastases by a primary tumour has been observed in laboratory animals (Gorelik et al., 1978) and defined as ‘concomitant immunity’ (Gorelik et al., 1981) or, more appropriately, ‘concomitant resistance’ (Meiss et al., 1986), a non-specific, rapid, dose-dependent and antigen-independent phenomenon. The molecular basis of concomitant resistance remains unknown, but it is speculated that several mechanisms may be acting either simultaneously or sequentially. It has been demonstrated in our laboratory that, although non-immunogenic, the LB tumour can induce concomitant resistance (Ruggiero et al., 1985), and that inhibition of secondary LB tumour growth by the presence of a primary tumour is abrogated if immunological Mφs are injected into the secondary site of tumour cell implantation (BustuOabad et al., 1984). We detected inhibition of cell migration induced by an LB tumour for a 4–5 day period, coinciding with the early stages of concomitant resistance. If our finding is correct, CI may therefore explain the early inhibition of growth of a secondary inoculum of LB cells; once the non-immunogenic neoplastic tissue is organised and vascularised, secondary tumour growth would not be affected by inhibition of Mφ migration induced by either CI or treatment with NSAIA.

Administration of different NSAIAAs as a single agent to inhibit tumour growth has been attempted (Humes et al., 1979, 1982; Narisawa et al., 1981; Panje, 1981; Pollard & Luckert, 1987; McCormick & Moon, 1983; Caignard et al., 1984; Fulton, 1984; Rubio, 1984; McCormick et al., 1985; Kort et al., 1986). Piroxicam treatment inhibits colon carcinogenesis (Pollard & Luckert, 1983; Reddy et al., 1987) in rats, and in combination with an anti-neoplastic agent it has also been used in man (Braun et al., 1987). The conflicting results obtained in these studies may be because of (i) a wide variety of spontaneous and induced tumours used experimentally; (ii) the tumours being transplanted in different sites; (iii) different animal species being used; (iv) NSAIA treatment being started at different times, before or after tumour challenge; and (v) NSAIA being administered alone or in combination with other drugs (Caignard et al., 1984). The major trend from these studies, however, is that NSAIA can inhibit tumour growth. Because high levels of PGE2 secreted by immunogenic tumours can interfere with immune responses against tumour cells (Normann et al., 1987), it has been postulated that the anti-tumour activity of NSAIAAs may be partly due to restoration of immune functions following inhibition of PGE2 synthesis (Lynch et al., 1978; Robertson et al., 1988). Other mechanisms which may explain the anti-tumour activity of NSAIAAs include inhibition of blood cell migration, as shown in this study, and the ability of NSAIAAs, including piroxicam, to form stable copper complexes (Weser et al., 1982), an important angiogenic factor necessary for neovascularisation (Ziche et al., 1982).

It can be concluded that a syngeneic tumour of non-detected immunogenicity can induce CI, which may play a role in the early stages of concomitant resistance, and that treatment with NSAIA delays LB tumour growth in vivo. Further studies are required to ascertain the usefulness of NSAIA treatment to prevent or delay the appearance of metastases.

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References

ATKINSON, D.C. & HICKS, R. (1975). The anti-inflammatory activity of irritants. Agents Actions, 5, 239.

ATTIA, M.A.M. & WEISS, D.W. (1966). Immunology of spontaneous mammary carcinomas in mice. V. Acquired tumour resistance and enhancement in strain. A mice infected with mammary tumour virus. Cancer Res., 26, 1787.

BARRERA, C.N., MAZZOLI, A.B., BUSTUOABAD, O.D., ANDRETTA, A.M., & PASQUALINI, C.D. (1985). Macrophages and tumour growth. II. Cell kinetics at the site of allogeneic tumour growth. Cancer Invest., 3, 7.

BENNET, A., HOUGHTON, J., LEAPER, D.J., & STAMFORD, I.F. (1979). Cancer growth, response to treatment and survival time in mice; beneficial effect of the prostaglandin synthesis inhibitor flurbiprofen. Prostaglandins, 17, 179.

BENNET, A., BATSTOCK, D.A., & CARROLL, M.A. (1982). Increased survival of cancer-bearing mice treated with inhibitors of prostaglandin synthesis alone or with chemotherapy. Br. J. Cancer, 45, 672.

BENNET, A., CARROLL, M.A., MELHUS, P.B., & STAMFORD, I.F. (1985). Treatment of mouse carcinoma in vivo with a prostaglandin E2 analogue and indomethacin. Br. J. Cancer, 52, 245.

BRAUN, D.P., BONOMI, P.D., TAYLOR, S.G., IV & HARRIS, J.E. (1987). Modification of the effects of cytotoxic chemotherapy on the immuno- and chemoresistance of cancer cells by a nonsteroidal anti-inflammatory drug, Piroxicam. A pilot study of the Eastern Cooperative Oncology Group. J. Biol. Response Mod., 6, 331.

BUSTUOABAD, O.D., GENOVESE, J.A., & PASQUALINI, C.D. (1984). Abrogation of concomitant tumour immunity in mice. Commun. Biol. (Buenos Aires), 2, 423.

CAIGNARD, A., MARTIN, M., REISSER, D., THOMAS, B. & MARTIN, F. (1984). Effects of cetimidine and indomethacin on the growth of dimethylhydrazone-induced or transplanted intestinal cancers in the rat. Br. J. Cancer, 50, 661.

FOLKMAN, J. (1985). Tumour angiogenesis. Adv. Cancer Res., 43, 175.

FOLKMAN, J. & COTRAN, R. (1976). Relation of vascular proliferation to tumour growth. Int. Rev. Exp. Pathol., 16, 207.

FULTON, A.M. (1984). In vivo effects of indomethacin on the growth of murine mammary tumours. Cancer Res., 44, 2416.

GOODWIN, J.S. & CEUPPENS, J. (1983). Regulation of the immune response by prostaglandins. J. Clin. Immunol., 3, 295.

GORELIK, E., SEGAL, S. & FELDMAN, M. (1981). On the mechanism of tumour ‘concomitant immunity’. Int. J. Cancer, 27, 847.

HUMES, J.L., CUPO, J.J., JR. & STRAUSSER, H.R. (1974). Effects of indomethacin on Melora sarcoma virus-induced tumours. Prostaglandins, 6, 463.

KORT, W.J.Z., HULSMAN, L.O.M., VAN SCHALKWIJK, W.P., WEIJ, I.M., ZONDERVERN, P.E. & WESTBROEK, D.L. (1986). Reductive effect of aspirin treatment on primary tumour growth and metastasis of implanted fibrosarcoma in rats. J. Natl Cancer Inst., 76, 711.

LYNCH, N.R., CASTES, M., ASTOIN, M. & SALOMON, J.C. (1978). Mechanism of inhibition of tumour growth by aspirin and indomethacin. Br. J. Cancer, 38, 503.

MCCORMICK, D.L., MADIGAN, M.J., JR. & MOON, R.C. (1985). Modulation of rat mammary carcinogenesis by indomethacin. Cancer Res., 45, 1803.

MCCORMICK, D.L. & MOON, R.C. (1983). Inhibition of mammary carcinogenesis by flurbiprofen, a non-steroidal anti-inflammatory agent. Br. J. Cancer, 48, 859.

MEISS, R.P., BONFIL, R.D., RUGGIERO, R.A. & PASQUALINI, C.D. (1986). Histologic aspects of concomitant resistance induced by nonimmunogenic murine tumours. J. Natl Cancer Inst., 76, 1163.
NARISAWA, T., SATO, M., TANI, M., KUDO, T., TAKAHASHI, T. & GOTO, A. (1981). Inhibition of development of methylnitrosourea-induced rat colon tumours by indomethacin treatment. Cancer Res., 41, 1954.

NORMANN, S.J., SCHARDT, M. & SORKIN, E. (1985a). Antileukocyte activity. I. Systemic inhibition of cellular emigration following local inflammation. J. Leukocyte Biol., 37, 319.

NORMANN, S.J., SCHARDT, M. & SORKIN, E. (1985b). Antileukocyte activity. II. Induction of tolerance to systemic anti-inflammation associated with local irritation and major surgery. J. Leukocyte Biol., 37, 331.

NORMANN, S.J., SCHARDT, M. & SORKIN, E. (1987). Cancer induced anti-inflammation and its potentiation by tumour excision and rechallenge. J. Leukocyte Biol., 42, 61.

NORMANN, S.J., BESEDOVSKY, H., SCHARDT, M. & DEL REY, A. (1988). Hormonal changes following tumor transplantation: factors increasing corticosterone and the relationship of corticosterone to tumour-induced anti-inflammation. Int. J. Cancer, 41, 829.

OYANAGUI, Y. (1984). Anti-inflammatory effects of polyamines in serotonin and carrageenan paw edema - possible mechanism to increase vascular permeability inhibitory protein level which is regulated by glucocorticoids and superoxide radical. Agents Actions, 14, 228.

PANIE, W.R. (1981). Regression of a head and neck carcinoma with a prostaglandin-synthesis inhibitor. Arch. Otolaryngol., 107, 658.

POLLARD, M. & LUCKERT, P.H. (1981). Effect of indomethacin on intestinal tumours induced in rats by the acetate derivative of dimethylnitrosamine. Science, 214, 558.

POLLARD, M. & LUCKERT, P.H. (1983). The suppressive effect of piroxicam on autochthonous intestinal tumours in the rat. Cancer Lett., 21, 57.

PREHN, R.T. & PREHN, L.M. (1987). The autoimmune nature of cancer. Cancer Res., 47, 927.

REDDY, B.S., MARUYAMA, H. & KELLOFF, G. (1987). Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a non-steroidal anti-inflammatory drug, during different stages of rat colon tumour development. Cancer Res., 47, 5340.

ROBERTSON, B., DOSTAL, K. & DAVIES, R.A. (1988). Neuropeptide regulation of inflammatory and immunologic responses. The capacity of alpha-melanocyte-stimulating hormone to inhibit tumour necrosis factor and IL-1-inducible biologic responses. J. Immunol., 140, 4300.

RUBIO, C.A. (1984). Antitumoral activity of indomethacin on experimental esophageal tumors. J. Natl Cancer Inst., 72, 705.

RUGGIERO, R.A., BUSTUOABAD, O.D., BONFIL, R.D., MEISS, R.P. & PASQUALINI, C.D. (1985). 'Concomitant immunity' in murine tumours of non-detectable immunogenicity. Br. J. Cancer, 51, 37.

SHIN, H.S., HAYDEN, M., LANGLEY, S., KALISS, N. & SMITH, M.R. (1975). Antibody-mediated suppression of grafted lymphoma. III. Evaluation of the role of thymic function, non-thymus-derivated lymphocytes, macrophages, platelets and polymorphonuclear leukocytes in syngeneic and allogeneic hosts. J. Immunol., 114, 1255.

SNYDERMAN, R., PIKE, M.C., BLAYLOCK, B.L. & WEINSTEIN, P. (1976). Effects of neoplasms on inflammation: depression of macrophage accumulation after tumour implantation. J. Immunol., 116, 585.

WESER, U., LENGFELDER, E., SELLINGER, K.H. & SCHOBOTZ, L. (1982). Reactivity of chelated copper with superoxide. In Inflammatory Diseases and Copper, Sorenson, J.R.J. (ed) p. 513. Humana Press: Clifton, NJ.

ZBAR, B., WEPSC, H.T., RAPP, H.J., STEWART, L.C. & BORSOS, T. (1970). Two-step mechanism of tumor graft rejection in syngeneic guinea pigs. II. Initiation of reaction by a cell fraction containing lymphocytes and neutrophils. J. Natl Cancer Inst., 44, 701.

ZICHE, M., JONES, J. & GULLINO, P. (1982). Role of prostaglandin E1 and copper in angiogenesis. J. Natl Cancer Inst., 69, 475.