The effect of adrenalectomy and dexamethasone on interleukin-1α induced responses in RIF-1 tumours

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Summary In the present studies the effect of bilateral adrenalectomy on the pathophysiologic responses to recombinant human interleukin-1α (rHIL-1α) was determined in RIF-1 tumour models. Acute vascular injury and haemorrhagic responses were quantitated by the intra-tumour accumulation of 59Fe radiolabelled erythrocytes. In vivo clonogenic tumour cell kill was determined by an excision assay. A single, intraperitoneal rHIL-1α treatment (6.25 × 10^7 Dₐ₀ units kg⁻¹, 25 μg kg⁻¹) resulted in acute tumour haemorrhage and approximately 35% clonogenic tumour cell kill (24 h). Bilateral adrenalectomy, 24 h before rHIL-1α, significantly increased haemorrhagic responses, but haemodynamic toxicity was severe. This toxicity could be ameliorated by giving dexamethasone (5 mg kg⁻¹) before or up to 3 h after rHIL-1α. The effect of dexamethasone on rHIL-1α induced tumour responses in adrenalectomised mice was sequence dependent. Given before rHIL-1α, dexamethasone inhibited tumour haemorrhage. When dexamethasone was given up to 3 h after rHIL-1α, tumour haemorrhage was directly related to sequence interval. Although adrenalectomy and dexamethasone alone had little effect on RIF-1 tumours, adrenalectomy increased rHIL-1α mediated clonogenic tumour cell kill. The surviving fraction 24 h after rHIL-1α (6.25 × 10^7 Dₐ₀ units kg⁻¹, 25 μg kg⁻¹) and dexamethasone (5 mg kg⁻¹, 2 h after rHIL-1α) was 1.3 ± 0.4%. The surviving fraction after this combination in intact mice (36.7 ± 1.4%) was approximately 30-fold higher than that seen in adrenalectomised mice. The results indicate that adrenal responses secondary to rHIL-1α treatment exert a negative feedback on rHIL-1α mediated responses in solid tumours.

Recombinant human interleukin-1α (rHIL-1α) has been shown to have significant anti-tumour activity in a variety of experimental tumour models (Nakamura et al., 1986; Nakata et al., 1988; Braunschweiger et al., 1988) and to be cytotoxic to some cell lines in vitro (Lachman et al., 1986). Although rHIL-1α was not toxic to RIF-1 tissue culture cells, this multifunctional cytokine had significant in vivo anti-tumour activity (Braunschweiger et al., 1988). In these later studies, rHIL-1α mediated anti-tumour responses were characterised by reduced tumour blood flow, intravascular congestion, extravascular haemorrhage, oedema, increased vascular permeability and clonogenic cell kill (Braunschweiger et al., 1988).

Adrenal hormones are thought to exert a negative feedback on the immune responses (del Rey et al., 1984). Naturally occurring and synthetic corticosteroid hormones are known to inhibit T cell mitogenesis (Gillis et al., 1979), natural killer cell (Holbrook et al., Gatti et al., 1986) and macrophage (Schaffner et al., 1985) function. Increases in plasma ACTH and corticosterone levels have been observed within 1 h after a single rHIL-1α treatment (Besedovsky et al., 1986; del Rey et al., 1987; Morrissey et al., 1988), and it was postulated that such increases might exert a negative feedback on rHIL-1α stimulated immune responses (del Rey et al., 1987). The possibility that this feedback pathway could be exploited, in therapeutic strategies, prompted the present studies to determine the role of adrenal hormones on rHIL-1α mediated anti-tumour activity.

Materials and methods

Tumour models

The RIF-1 fibrosarcoma tumour model was maintained according to the protocol described by Twentyman et al., (1980). RIF-1 cells were propagated in RPMI 1640 medium (Mediatech, Washington DC) supplemented with 10% fetal bovine serum (FBS) (Irvine Scientific, Santa Ana, CA) and 2 mM glutamine (Gibco, Grand Island, NY), as described previously (Braunschweiger et al., 1982; Braunschweiger & Schiffer, 1986). RIF-1 tumours were routinely produced in 6–10 week old (~ 20 g), endotoxin hyposensitive, female C3H/Hej mice (Jackson Laboratories, Bar Harbor, ME) by inoculating 5 × 10⁶ tissue culture cells, subcutaneously, on the right flank. Studies were initiated 14 days later when tumours weighed approximately 0.5 g.

Mice were quarantined for 2 weeks before entering studies. Randomly selected mice were tested and found to be free of adventitious murine viruses. All mice were housed, 4–5 per cage, in a temperature and humidity controlled facility with a 12 h light–dark cycle (lights on at 0600 local time) accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). Mice were provided standard mouse chow and water ad libitum. All procedures were reviewed and approved by the Animal Care and Use Committee of the AMC Cancer Research Center.

Interleukin-1α

Recombinant human interleukin-1α was generously provided by Dr Peter Lomedico (Hoffman-LaRoche, Nutley, NJ). The rHIL-1α used in our studies was highly purified (2.5 × 10⁷ units mg⁻¹ protein, D₀ assay) and essentially free of endotoxin contamination (<0.125 EU mg⁻¹ protein, LAL assay). rHIL-1α was diluted in pyrogen free, sterile 0.9% NaCl containing 0.05% BSA and administered by intraperitoneal injection in 0.2 ml of vehicle. Our standard mouse dose (6.25 × 10⁷ D₀ units kg⁻¹, 25 μg kg⁻¹) for these studies was less than 5% of the LD₉₀ dose for intact mice. This dose is 2–3 times that shown by Johnson et al. (1989) to stimulate haematopoiesis sub-optimally in normal mice. Control mice received vehicle alone as previous studies indicated that heat inactivated rHIL-1α had no activity at this dose levels. All RIF-1α and vehicle injections were made at 0800–0900 local time.

Adrenalectomy

Bilateral adrenalectomy (ADX) was performed, via the dorsal route, using Metafene (Pittman-Moore Inc., Washington Crossing, NJ) anaesthesia, 13 days after tumour inoculation. rHIL-1α treatments were initiated on day 14. Adrenalectomised mice were given 0.9% NaCl drinking water ad

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libitum. In some experiments, dexamethasone (dexamethasone sodium phosphate; ESI Pharmaceuticals Inc.) was administered as 5 mg kg\(^{-1}\) in 0.2 ml sterile, non-pyrogenic 0.9% NaCl by intraperitoneal injection.

**Quantitative end-points**

The haemorrhagic response in RIF-1 tumours after rHIL-la was quantitated by determining the tumour packed erythrocyte volume using a \(^{59}\)Fe-RBC dilution method described previously (Braunschweiger et al., 1988; Braunschweiger & Schiffer, 1986). One µCi of \(^{59}\)Fe-citrate (NEN, Boston, MA) was injected, i.p., 7 days after tumour implantation. Studies were initiated at least 7 days later to provide adequate time for labelled erythrocytes to enter circulation and for unincorporated \(^{59}\)Fe to be cleared (Alpen & Cranmore, 1959). At various intervals after RIF-1la treatment, peripheral venous blood was obtained in heparinised hematocrit tubes from the post-orbital venous plexus and centrifuged. The \(^{59}\)Fe radioactivity in the packed RBC fraction was determined in a gamma well counter (Packard Instruments, Downers Grove, IL) and expressed as a percentage of the injected dose (ID) per microlitre of packed RBCs (%ID µl\(^{-1}\)). After obtaining the blood sample, the mice were killed by cervical luxation. The tumours were resected in toto and weighed. The \(^{59}\)Fe radioactivity was determined, as above, and expressed as the %ID g\(^{-1}\) wet weight. The tissue packed RBC volume (µl g\(^{-1}\)) was calculated as described previously (Braunschweiger & Schiffer, 1986).

Clonogenic cell survival in RIF-1 tumours was assessed by a modification (Braunschweiger et al., 1982, 1983, 1988) of the excision clonogenic cell survival assay described originally by Twentyman et al. (1980), 24 h after RIF-1la treatment. Control tumour cell yields were routinely 7–14 × 10\(^5\) trypan negative cells g\(^{-1}\).

**Statistical analysis**

Analysis of variance was used to assess overall variation and the significance of post-treatment responses (Snedecor & Cochran, 1980). The Newman–Keuls multiple range test was used to determine the significance between treatment group means (Zar, 1974). Where appropriate, Student’s t test was also used. A P value of less than, or equal to, 0.05 was considered adequate justification to reject the null hypothesis.

**Results**

In C3H/HeJ mice untreated subcutaneous RIF-1 tumours of less than 1 g rarely exhibit surface haemorrhagic, skin ulcerations, central or even local necrosis. A single intraperitoneal injection of 1 × 10\(^7\) units kg\(^{-1}\) of rHIL-la (6.25 × 10\(^5\) units kg\(^{-1}\); 25 µg kg\(^{-1}\)) resulted in acute haemorrhagic responses that were visibly obvious within 3–6 h after treatment. Histologically, progressive vascular congestion (within 1 h) and frank vascular breakdown and haemorrhage were seen by 4 h after treatment. In ADX mice a single injection of 1 × 10\(^7\) units kg\(^{-1}\) of rHIL-la (6.25 × 10\(^5\) units kg\(^{-1}\); 25 µg kg\(^{-1}\)) resulted in haemodynamic shock and the death of 5/5 mice by 12 h after treatment. The haemodynamic shock and systemic toxicity of rHIL-la in ADX C3H/HeJ mice was ameliorated by giving dexamethasone (5.0 mg kg\(^{-1}\)) within 3 h after rHIL-la treatment. At 24 h after rHIL-la treatment, the haemorrhagic response in RIF-1 tumours in ADX mice (dexamethasone, 5 mg kg\(^{-1}\), 2 h after rHIL-la) was qualitatively greater than that seen in intact mice treated with rHIL-la alone (Figure 1). This haemorrhagic response was quantitated by the intra-tumour accumulation of \(^{59}\)Fe-RBC.

The effect of ADX and dexamethasone on the rHIL-la mediated haemorrhagic responses was quantitated by determining changes in the tumour packed RBC volumes (Figure 2). Bilateral ADX was performed 24 h before rHIL-la, and changes in tumour packed RBC volumes were determined 6 h after rHIL-la. This study interval was chosen because an equilibrium is seen by 6 h (Braunschweiger et al., 1988) and, as stated above, haemodynamic toxicity after rHIL-la in ADX mice precluded longer study intervals unless dexamethasone was also administered. Analysis of variance indicated that treatment group means were not derived from the same sample population (F = 19.6; P < 0.0001). In these studies, dexamethasone (5 mg kg\(^{-1}\)) alone, ADX alone or ADX plus dexamethasone produced no visual haemorrhage and had no effect on tumour packed RBC volumes. rHIL-la alone significantly (P < 0.05) increased tumour packed RBC volumes in both intact mice and ADX mice. The 6 h response in ADX mice was significantly (P < 0.05) greater than that seen in intact mice. Dexamethasone given to ADX mice, 30 min before rHIL-la, prevented tumour haemorrhage at 6 h. When dexamethasone was given after rHIL-la, the magnitude of the tumour haemorrhage in ADX mice (6 h) was sequence dependent. The packed RBC volumes, observed when dexamethasone was given 2 or 3 h after rHIL-la, were greater than those seen in intact controls after rHIL-la alone or after rHIL-la and dexamethasone (2 h after rHIL-la). When dexamethasone was given to ADX mice before or up to 3 h after rHIL-la, no mortality was observed for up to 24 h after treatment. Longer observation periods were not studied.

Figure 3 shows the time dependent changes in packed RBC volumes for tumours in intact and adrenalectomised mice treated with rHIL-la and dexamethasone (5 mg kg\(^{-1}\), 2 h after rHIL-la). The haemorrhagic response in RIF-1 tumours in intact mice treated with the rHIL-la, dexamethasone combination was not statistically different from that seen after rHIL-la alone. However, the haemorrhagic response in tumours from adrenalectomised mice treated with the combination of rHIL-la and dexamethasone (2 h after rHIL-la) was greater, at all study intervals, than that seen after a similar combination in intact mice. Similar time course studies in ADX mice treated with rHIL-la alone could not be conducted at the same rHIL-la dose level due to severe haemodynamic toxicity.

Table 1 shows the results from a representative experiment to determine the effect of ADX and dexamethasone on clonogenic cell survival (24 h) in RIF-1 tumours. rHIL-la alone resulted in 45% clonogenic cell kill. Although adrenalectomy had no significant effect on clonogenic cellularity, the combination of rHIL-la and dexamethasone (2 h
The effect of adrenalectomy (A), dexamethasone (D) and rHIL-1α (6.25 × 10^5 Dₐ₀ units kg⁻¹) alone and in combination on packed RBC volumes of the RIF-1 tumours. Adrenalectomy was performed 24 h before rHIL-1α and packed RBC volumes were determined 6 h after rHIL-1α. Dexamethasone (5 mg kg⁻¹) was given 2 h after rHIL-1α in intact mice (IL-1α + D), 24 h after adrenalectomy (A + D), 3 h before rHIL-1α (A + D + IL-1α) or at 0.5, 1.0, 2.0 or 3.0 h after rHIL-1α in adrenalectomised mice. Each bar represents the mean ± 1 s.d. for six tumours. ANOVA indicated significant treatment effects and the Newman–Keuls multiple range test indicated that the response to rHIL-1α alone in intact mice was significantly different (*) from that seen in all other groups except those seen in intact mice treated with rHIL-1α + D and those in adrenalectomised mice treated with dexamethasone 30 min or 1 h after rHIL-1α.

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Figure 2: The effect of adrenalectomy (A), dexamethasone (D) and rHIL-1α (6.25 × 10^5 Dₐ₀ units kg⁻¹) alone and in combination on packed RBC volumes of the RIF-1 tumours. Adrenalectomy was performed 24 h before rHIL-1α and packed RBC volumes were determined 6 h after rHIL-1α. Dexamethasone (5 mg kg⁻¹) was given 2 h after rHIL-1α in intact mice (IL-1α + D), 24 h after adrenalectomy (A + D), 3 h before rHIL-1α (A + D + IL-1α) or at 0.5, 1.0, 2.0 or 3.0 h after rHIL-1α in adrenalectomised mice. Each bar represents the mean ± 1 s.d. for six tumours. ANOVA indicated significant treatment effects and the Newman–Keuls multiple range test indicated that the response to rHIL-1α alone in intact mice was significantly different (*) from that seen in all other groups except those seen in intact mice treated with rHIL-1α + D and those in adrenalectomised mice treated with dexamethasone 30 min or 1 h after rHIL-1α.

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Figure 3: The time dependent changes in packed RBC volumes in RIF-1 tumours after rHIL-1α (6.25 × 10^5 Dₐ₀ units kg⁻¹) and dexamethasone (5 mg kg⁻¹, 2 h after rHIL-1α) in intact mice (●, n = 13) and in mice adrenalectomised 24 h before the rHIL-1α and dexamethasone treatment (▲, n = 6). Also shown are data for tumours in intact mice treated with IL-1α alone (■, n = 6). Each symbol is the mean ± 1 s.d.

![Figure 4](image_url)

Figure 4: The effect of rHIL-1α (6.25 × 10^5 Dₐ₀ units kg⁻¹) alone, or in combination with adrenalectomy and dexamethasone, on clonogenic cell survival in RIF-1 tumours. a, dexamethasone (5 mg kg⁻¹), two experiments with four tumours; b, rHIL-1α alone, eight experiments with 19 tumours; c, dexamethasone (5 mg kg⁻¹) given 30 min before rHIL-1α in intact mice, two experiments with four tumours; d, dexamethasone (5 mg kg⁻¹) given 2 h after rHIL-1α in intact mice, two experiments with four tumours; e, adrenalectomy alone, three experiments with eight tumours; f, dexamethasone given 30 min before rHIL-1α in adrenalectomised mice, one experiment with three tumours; g, dexamethasone given 2 h after rHIL-1α in adrenalectomised mice, six experiments with 14 tumours. Clonogenic cell survival assays was determined by excision 24 h after rHIL-1α or dexamethasone treatment. In each experiment, treated and control tumours were concurrently studied. Tumour tissue from two to three mice was pooled for clonogenic assays in each experiment. Each bar represents the mean ± 1 s.e. *Significantly (P < 0.05) different from rHIL-1α alone by ANOVA and the Newman–Keuls multiple range test.
1.27 ± 0.36% (g). This decreased survival is highly significant as compared to rHIL-1α alone (P < 0.0005) or the same combination (d, P < 0.002) in intact mice. RIF-1 clonogenic cellularity after dexamethasone (a) or adrenalectomy alone (e) was significantly greater than that seen after rHIL-1α in intact mice (b). In intact mice, rHIL-1α and dexamethasone (2 h after rHIL-1α; d) produced a surviving fraction (36.7 ± 1.4%) consistent with the additive effects of rHIL-1α and dexamethasone alone. When dexamethasone was given 30 min before rHIL-1α, clonogenic cell survival in ADX (f) and intact (c) were similar. Our results suggest that the post-rHIL-1α adrenal response not only protects the host from haemodynamic toxicity but also inhibits anti-tumour responses mediated by rHIL-1α.

**Discussion**

Our data suggest that adrenal hormones play a major role in controlling rHIL-1α mediated toxicity and anti-tumour activity. Although prior ADX greatly increased haemorrhagic responses in tumours after rHIL-1α, haemodynamic toxicity was severe. Recent studies indicated that dexamethasone treatment before rHIL-1α or TNF could ameliorate the haemodynamic toxicity of these cytokines in adenalecotomised CD-1 mice (Bertini et al., 1988). We have not only confirmed these findings in the C3H/HeJ mouse, but also demonstrated that dexamethasone up to 3 h after rHIL-1α prevented toxic deaths. Dexamethasone given before rHIL-1α not only prevented haemodynamic toxicity in ADX mice, but also inhibited rHIL-1α induced haemorrhagic responses in RIF-1 tumours. On the other hand, when dexamethasone was given after rHIL-1α, haemorrhagic responses increased as the sequence interval increased, up to 3 h.

Adrenalectomy not only enhanced rHIL-1α mediated vascular responses, but also increased clonogenic tumour cell kill after rHIL-1α. In intact mice, rHIL-1α alone and rHIL-1α plus dexamethasone resulted in 46% and 36% surviving fractions, respectively. We previously showed that although dexamethasone may have profound anti-proliferative effects in RIF-1 tumours (Braunschweiger et al., 1982, 1983; Braunschweiger & Schiffer, 1986), dose levels much higher than those used here produced little tumour cell kill (Braunschweiger et al., 1982). In the present studies, the surviving fraction after a single 5 mg kg⁻¹ dexamethasone treatment was 78%. Since the additive effects of the dexamethasone and rHIL-1α treatments would be expected to yield approximately 35% survival, the observed 12.7% surviving fraction for RIF-1 tumours in adrenalectomised mice treated with rHIL-1α and dexamethasone (5 mg kg⁻¹, 2 h after rHIL-1α; group g) would indicate that adrenal responses within 2 h after rHIL-1α have a direct or indirect antagonistic effect on rHIL-1α anti-tumour activity. This is further supported by the observation that dexamethasone given before rHIL-1α in ADX mice (group f) inhibited rHIL-1α and mediated clonogenic cell kill.

In C3H/HeJ mice, plasma ACTH and corticosterone were increased within 1 h of rHIL-1α treatment (Besedovsky et al., 1986; del Rey et al., 1987; Morrissey et al., 1988). Corticosteroids are known to inhibit immune functions (Gillis et al., 1979; Holbrook et al., 1983; Gatti et al., 1986; Schaffner, 1985) and corticosteroid associated immunoregulation has been proposed as a homeostatic surveillance mechanism for the control of immune cell function (del Rey et al., 1984, 1987). While this might explain the enhanced rHIL-1α anti-tumour responses in adrenalectomised mice, other mechanisms could be operative. rHIL-1α is known to promote the adherence of leukocytes to endothelial cells (Bevilacqua et al., 1985). Since rHIL-1α can stimulate leukocytes to release proteolytic enzymes and hydrogen peroxide (Ozaki et al., 1987) such responses could contribute to rHIL-1α mediated tumour vascular injuries. Further, specific high affinity glucocorticoid receptors have been demonstrated in cultured endothelial cells (Lewis et al., 1988), and corticosteroids inhibit neutrophil (Ebert & Barclay, 1952), granulocyte (MacGregor, 1976) and lymphoid cell (Maca et al., 1978) adherence to the endothelium.

rHIL-1α is also known to stimulate plasminogen activator inhibitor (Nachman et al., 1986), procoagulant activity (Bevilacqua et al., 1983) and arachidonic acid metabolism (Bernheim, 1986; Besedovsky et al., 1975; Dinarello et al., 1983). Prostaglandins (PG) E₂ and I₂ are potent vasodilators which can increase capillary permeability (Kuehl & Egner, 1980; Issekutz & Movat, 1983). Lipoxygenase metabolites are also vasoactive (Letts & Cirino, 1985), increase vascular permeability and promote granulocyte adherence (Samuelson, 1983). Corticosteroids are well known inhibitors of PG and leukotriene synthesis. The concentration of hydrocortisone needed to produce near maximal inhibition of endothelial cell PG synthesis in vitro (Lewis et al., 1986) was similar to the corticosterone concentrations measured in the sera of mice after rHIL-1α treatment (Besedovsky et al., 1986; del Rey et al., 1987). These findings might indicate an important role for vasoactive arachidonic acid metabolites in the rHIL-1α induced ischaemia and clonogenic cell kill in RIF-1 tumours.

The responses to rHIL-1α in RIF-1 tumours are not unlike those seen in tumours treated with tumour necrosis factor (Watanabe et al., 1988; Havell et al., 1988). rHIL-1α and TNF may have similar activities (Bevilacqua et al., 1983) and/or be synergistic (Last-Barney et al., 1988; Ruggiero & Baglioni, 1987). Further, the regulation of rHIL-1α and TNF production by monocytes and macrophages may be closely interrelated (Philip & Epstein, 1986), and recent evidence suggests that these cytokines may be intimately involved in local macrophage mediated remodelling of tissue extracellular matrix (Vlassara et al., 1988). Although it is tempting to postulate a role for TNF in rHIL-1α mediated tumour responses, there is as yet no direct evidence, aside from similar pathophysiology, to support the attractive hypothesis that TNF may be a local mediator of rHIL-1α responses in RIF-1 tumours.

Since the profound vascular injury which characterised rHIL-1α responses in RIF-1 tumours was not seen in muscle and skin of intact (Braunschweiger et al., 1988) or adrenalectomised hosts, the differences between tumour and normal tissue vascular structure, function and/or regulation leading to rHIL-1α induced tumour vascular injury might provide a focus for the development of new therapeutic strategies. Studies, currently underway, to investigate the negative feedback by adrenal hormones on rHIL-1α mediated tumour and host cell responses could lead to new approaches to enhance the anti-tumour activity of this multifunctional cytokine. In this regard, agents which transiently block adrenal steroid hormone synthesis (e.g. ketoconazole) potentiate rHIL-1α anti-tumour activity (Braunschweiger et al., 1989) without attendant increases in toxicity.

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