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Effects of micro-environment- and malignant cell-derived interleukin-1 in carcinogenesis, tumour invasiveness and tumour–host interactions

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ABSTRACT

Interleukin-1 (IL-1) comprises a family of closely related genes; the two major agonistic proteins, IL-1α and IL-1β, are pleiotropic and affect mainly inflammation, immunity and haemopoiesis. IL-1β is active solely in its secreted form, whereas IL-1α is active mainly as an intracellular precursor. IL-1 is abundant at tumour sites, where it may affect the process of carcinogenesis, tumour growth and invasiveness and the patterns of tumour–host interactions. Here, we review the effects of micro-environment- and tumour cell-derived IL-1 on malignant processes in experimental tumour models. We propose that membrane-associated IL-1α expressed on malignant cells stimulates anti-tumour immunity, while secretable IL-1β derived from the micro-environment or the malignant cells, activates inflammation that promotes invasiveness and induces tumour-mediated suppression. Inhibition of the function of IL-1 by the inhibitor of IL-1, interleukin-1 receptor antagonist (IL-1Ra), reduces tumour invasiveness and alleviates tumour-mediated suppression, pointing to its feasible use in cancer therapy. Differential manipulation of IL-1α and IL-1β in malignant cells or in the tumour's micro-environment may open new possibilities for using IL-1 in cancer immunotherapy.

1. Introduction

The association of cancer with chronic inflammation has been recognised for some time and recently has become the focus of experimental tumour systems (reviewed in Refs. 1–10). Persistent expression of pro-inflammatory cytokines at tumour sites may exert pleiotropic effects, ranging from increasing the invasiveness of the malignant cells to activation of anti-tumour immune surveillance mechanisms that have the potential to eradicate the malignant cells or inhibit tumour growth. Of special relevance to the malignant process are interleukin-1 (IL-1) and tumour necrosis factor (TNF)-α, which are considered 'alarm cytokines'; they are generated by macrophages immediately after confronting the inflammatory stimuli. IL-1 and TNF-α cause inflammation, but more importantly, they induce the expression of pro-inflammatory genes in diverse stromal/inflammatory cells, which results ultimately in a local cascade of cytokines and small effector molecules that propagate and sustain inflammation. Of major importance are cyclo-oxygenase type 2 (COX-2), inducible nitric oxide synthase (iNOS), IL-6 and other cytokines/chemokines. In addition, IL-1 and TNF-α increase the expression of

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adhesion molecules on endothelial cells and leukocytes, which promote leukocyte infiltration from the blood into tissues.

At tumour sites, IL-1 is abundant, being produced and secreted by the malignant cells or cellular elements of the micro-environment, in response to local inflammatory signals. IL-1 has pleiotropic effects on distinct phases of the malignant process, such as carcinogenesis, tumour invasiveness and patterns of interactions between the malignant cells and the defence mechanisms of the host. This review describes the plethora of effects of IL-1 on malignant processes, mainly emphasising experimental systems that have been studied in our laboratory.

2. The interleukin-1 family

Three gene products of this family have been studied thoroughly: two agonistic proteins, namely IL-1α and IL-1β, and one antagonistic protein, the IL-1 receptor antagonist (IL-1Ra). IL-1Ra, which binds to IL-1 receptors without transmitting an activation signal, represents a physiological inhibitor of pre-formed IL-1 (reviewed in Refs. 11–20).

IL-1α and IL-1β are synthesised as 31 kDa precursors, which are further processed by proteases to their mature 17 kDa forms. IL-1β-converting enzyme (ICE), or caspase-1, is a cysteine protease that cleaves the inactive precursor of IL-1β, while proIL-1α is processed by calpain.

IL-1 differs from most other cytokines by the lack of a signal sequence, thus not passing through the endoplasmic reticulum (ER)–Golgi pathway; its mechanism of secretion is not yet completely understood. IL-1Ra has a signal peptide and is secreted in the ER–Golgi exocytic pathway. Non-secretable intracellular isoforms of the IL-1Ra (icIL-Ra), lacking a signal peptide, were also described; it was suggested that they neutralise the active intracellular precursor of IL-1α (proIL-1α).

Many cell types produce and secrete IL-1α, IL-1β and IL-1Ra upon activation with microbes, microbial products, cytokines and other environmental stimuli. Mononuclear cells secrete the highest levels of IL-1α and IL-1β, while non-phagocytic cells secrete IL-1 in only a very limited fashion, mainly IL-1β. As IL-1α is secreted to a much lesser extent than IL-1β, even in activated monocytes, it is not commonly detected in body fluids, except in severe inflammatory responses, in which case it is probably released by necrotising cells.

In their recombinant form, IL-1α and IL-1β bind to the same receptors and exert the same biological activities. However, IL-1α and IL-1β differ in various aspects. In comparison with IL-1α, IL-1β expression and secretion is tightly controlled at the levels of transcription, mRNA stability, translation, processing and secretion. Also, IL-1α and IL-1β differ dramatically in the sub-cellular compartments in which they are active. IL-1β is solely active as a secreted product, while IL-1α is active as an intracellular precursor, as a membrane-associated form (23 kDa), but is only marginally active in its secreted mature form, due to its limited secretion. The active membrane form of IL-1α is derived from mirysoylation of proIL-1α and it is anchored to the membrane via a mannose-like receptor.

We have hypothesised that the localisation of the IL-1 molecules in the context of the producing cell and its micro-environment dictates their biological functions (see Fig. 1) (reviewed in Ref. 12). Thus, as is shown below, membrane-associated IL-1α is immunostimulatory,[21–27] while cytosolic proIL-1α may control homeostatic functions, such as gene expression, proliferation control and differentiation.[28] As to secreted IL-1 (mainly IL-1β), at low local doses, it induces limited inflammatory responses followed by activation of specific

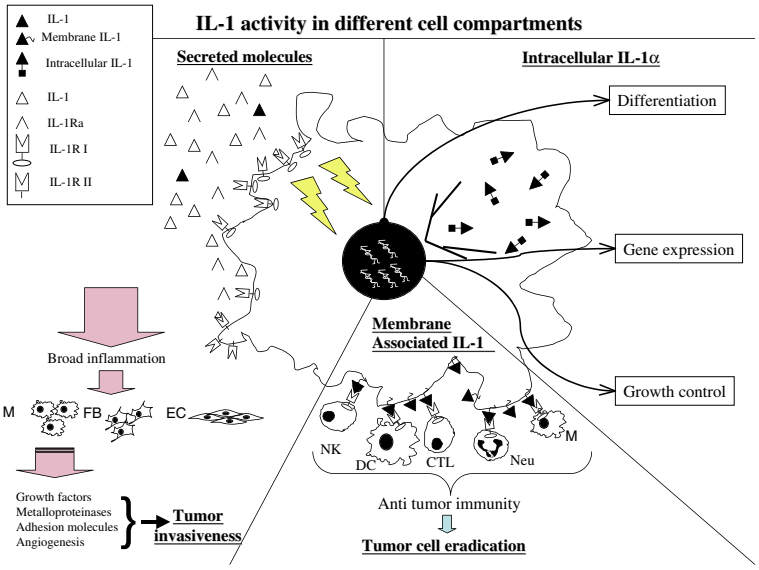

Fig. 1 – IL-1 activity in different sub-cellular compartments in malignant cells. In cells which express the precursor of interleukin (IL)-1α, active cytosolic and membrane-associated IL-1α can be detected. In IL-1β-expressing cells, the cytokine is secreted into the micro-environment following processing by caspase-1. Shown in the scheme are also the interleukin-1 receptors (IL-1Rs): the IL-RI signalling receptor and the components that down-regulate the function of pre-formed IL-1, i.e., the interleukin-1 receptor antagonist (IL-1Ra) and the decoy receptor, IL-1RII. Similar patterns of IL-1 expression and control mechanisms are also seen in primary cells.

immune mechanisms, while at high doses, broad inflammation accompanied by tissue-damage and tumour invasiveness are evident.[25,27] In addition, we have demonstrated that in vivo, in steady-state homeostasis and in inflammation, IL-1α and IL-1β are differentially expressed in tissues, possibly pointing to their different physiological roles.[29,30]

IL-1 receptors (IL-1Rs), which belong to the immunoglobulin (Ig) supergene family, are abundantly expressed on many cell types. IL-1R of type I (IL-1RI) (80 kDa) is a signalling receptor, whereas the IL-1R of type II (IL-1RII) (68 kDa) serves as a decoy target, acting to reduce excessive amounts of IL-1 (reviewed in Refs. 11–20). Following the binding of IL-1 to IL-1RI, a second chain, i.e., the IL-1R acceptor protein (IL-1RAcP), is recruited. This heterodimeric complex triggers IL-1 signal transduction, which is initiated by activation of the IL-1 receptor-associated kinase (IRAK) and ultimately leads to activation of nuclear genes through the activation of NF-κB and other transcription factors. IL-1RII and the IL-1Ra fail to form the heterodimeric complex with the IL-1AcP and to recruit IRAK. Thus, under physiological homeostasis, IL-1Ra, surface IL-1RtII and soluble IL-1Rs play a key role in limiting cell responsiveness to IL-1.

Knockout (KO) mice that are deficient in genes that encode for proteins of the IL-1 family have served as an important tool to delineate the role of these proteins. A series of KO mice that are deficient in different genes of the IL-1 family has been generated by the group of Y. Iwakura[31,32]; this has enabled the delineation of differences in the functions of IL-1β and IL-1α in health and disease. Mice deficient in IL-1 are normally born healthy and mature, while mice deficient in IL-1Ra have small litters and exhibit growth retardation in adult life. Inflammatory responses were shown to be attenuated in IL-$1\beta^{-/-}$ mice, non-impaired in IL-$1\alpha^{-/-}$ mice, and accentuated in IL-$1Ra^{-/-}$ mice. In IL-$1Ra^{-/-}$ mice of BALB/c background, a chronic inflammatory polyarthropathy, which resembles the pathology of human rheumatoid arthritis, develops spontaneously.[32,33] In addition, IL-$1Ra^{-/-}$ mice develop lethal arterial inflammation at arterial bifurcations, where atherosclerotic plaques are commonly found.[34] Further studies on the role of IL-1β and IL-1α in controlling specific immune responses have revealed that IL-1β is more important for the manifestation of antibody responses,[35,36] while IL-1α is probably more dominant in the control of delayed type hypersensitivity (DTH),[37] a phenomenon of cell-mediated immunity.

3. Effects of IL-1 on chemical carcinogenesis

Most information on the role of the IL-1 molecules in the malignant process concerns the effects of IL-1 on invasiveness of already existing malignant cells; in cancer patients, increased local levels of IL-1 usually correlates with tumour invasiveness and a poor prognosis (reviewed in Ref. 12). Little is known about the effects of the IL-1 molecules on the process of carcinogenesis.

In our studies we have used an experimental system of in vivo chemical carcinogenesis, induced by 3-methylcholantrene (3-MCA), which simulates human tumour development following exposure to repetitive environmental insults. By applying 3-MCA to control and IL-1 KO mice, i.e., IL-$1\alpha^{-/-}$, IL-$1\beta^{-/-}$, IL-$1\alpha/\beta^{-/-}$ (double KO mice) or IL-$1Ra^{-/-}$ mice, the role of host-derived IL-1 molecules in the susceptibility to chemical carcinogenesis was assessed. Fibrosarcomas develop in carcinogen-treated mice after about 3–4 months. In this model, 3-MCA acts as a complete carcinogen, executing the functions of both the initiator and the tumour promoter. Initiation of carcinogenesis involves the initial induction of genetic changes in target cells, whereas during tumour promotion, the affected cells are rescued from apoptosis, stimulated to proliferate and accumulate more mutations, which ultimately leads to the development of overt malignant cells that form tumours.

Our results have highlighted the role of secretable microenvironment-derived IL-1β in the development of chemically induced tumours (see Fig. 2). In mice deficient in IL-1β, i.e., in IL-$1\beta^{-/-}$ and IL-$1\alpha/\beta^{-/-}$ (double KO), 3-MCA-induced tumours started to develop only after a prolonged lag period (about 110 d), while in control wild-type mice and IL-$1\alpha^{-/-}$ mice, most mice developed tumours that appeared earlier (around 80 d). In IL-$1Ra^{-/-}$ mice, in which unattenuated levels of the IL-1 molecules exist, tumour development is the most rapid and all mice developed tumour around d 115. Thus, while the role of IL-1β in promoting chemical-induced carcinogenesis is pronounced, the role of IL-1α, which is mainly cell-associated, is less evident in this model of carcinogenesis.

IL-1 may be involved in the initial stages of carcinogenesis, in the process of mutagenesis by activating infiltrating phagocytes or the target cells for transformation to produce mutagenic ROI or NOI. Following the initial mutagenic effect, local IL-1 may further propagate the malignant process by stimulating proliferation of the pre-malignant cells in either an autocrine or paracrine manner. Extensive proliferation leads to the accumulation of mutations in the pre-malignant cells. IL-1 may also enhance the invasiveness of already existing tumour cells by switching on angiogenesis and by the induction of inflammatory molecules, such as matrix

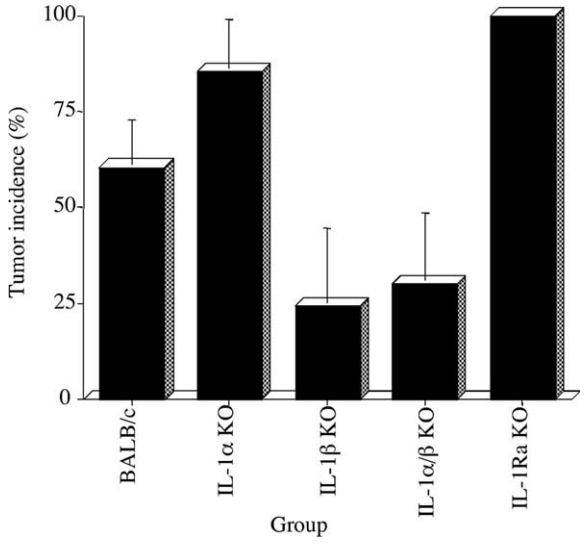

Fig. 2 – Tumour incidence in carcinogen-treated IL-$1^{-/-}$ and control mice. Mice were injected with 3-methylcholantrene in olive oil (3-methylcholantrene (3-MCA), 200 μg/mouse intra-muscularly) and tumour incidence on d 115 was determined.

metalloproteinases (MMPs), heparanase, chemokines or integrins on the malignant cells or endothelial cells, leading to tumour dissemination and metastasis.

Most interesting is the type of tissue reaction that is observed at the site of carcinogen application and which may pronouncedly affect the malignant process. Blankenstein's group has recently described a mechanism of carcinogen encapsulation by a foreign body-like granuloma that consists of fibroblasts that form a scar tissue around droplets of the carcinogen.[38–40] This foreign body reaction inhibits the diffusion of the carcinogen into the tissue; this diminishes tissue injury, protects from DNA damage and ultimately inhibits tumour development. This phenomenon can be experimentally demonstrated in mice injected with very low doses of 3-MCA, where reduced tumour incidence is observed. In IFN$\gamma^{-/-}$ and IFNγR$^{-/-}$ mice, this mechanism is impaired and mice are more susceptible to chemical-induced carcinogenesis.[38–40] This indicates that IFNγ is involved in the formation of this fibrotic capsule, by mechanisms that are not yet completely understood.

In our studies, we have observed that in control, IL-1$\alpha^{-/-}$, IL-1$\beta^{-/-}$ and IL-1$\alpha/\beta^{-/-}$ mice, droplets of the carcinogen in oil are encapsulated by fibrotic tissue, mainly consisting of fibroblasts and extracellular matrix (ECM) fibres, with very little infiltration of leukocytes (see Fig. 3(A)). On the contrary, in IL-1Ra$^{-/-}$ mice, large multilayer granulomas surround the lipid droplets containing the carcinogen. These granulomas are rich in phagocytes, mainly neutrophils, but also macrophages with the morphology of foam cells, and fibroblasts (see Fig. 3(B)). Almost no lymphocytes were observed in these granulomas, indicating their innate nature. Immunohistochemical staining revealed that the granulomas consist of cells expressing pro-inflammatory molecules, such as IL-1β, COX-2, and to a lesser extent IL-1α and TNF-α. The presence of this type of infiltrate is evident from d 10 after 3-MCA injection; it persists until the development of overt tumours. Such inflammatory responses may enhance the process of tumourigenesis. 3-MCA activates the expression of IL-1 and other pro-inflammatory molecules in phagocytes and thus amplifies the action of the carcinogen and propagates the process of tumourigenicity.

The importance of micro-environment-derived TNF-α in the development of malignancies, was demonstrated recently in experimental systems of skin 7,12-dimethylbenzantracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA)[41–46] and liver[47] carcinogenesis, where decreased tumour incidence was observed in TNF-$\alpha^{-/-}$ and TNFR$^{-/-}$ mice. In a model of skin carcinogenesis (DMBA/TPA), the local expression of TNF-α at the site of application of the tumour promoter (TPA) activates expression of genes that are important for tumour growth/invasiveness, e.g., GM-CSF, MMP-9 and MMP-3.[41–46] 'Residual' skin tumours that developed in TNF-$\alpha^{-/-}$ mice, were attributed to the pro-inflammatory function of IL-1 that is induced in the skin by the tumour promoter TPA.[44] Up-regulation of IL-1α was also observed in the skin of mice inoculated with DMBA alone, indicating that IL-1 is involved in both tumour initiation and promotion.[48] In an experimental system of skin tumour induction, in a two-step carcinogenesis model (DMBA/TPA), we have observed an increase in the rate of development of papillomas in mice expressing unopposed levels of IL-1, i.e., IL-1Ra$^{-/-}$ mice, compared with control mice or mice deficient in IL-1 molecules (Reich and colleagues). Histologically, skin crusts, heavily infiltrated by neutrophils, were abundant in the skin of DMBA/TPA-treated IL-1Ra$^{-/-}$mice. Thus, supra-physiological levels of IL-1 contribute to the promotion of inflammation-mediated tumourigenesis. On the other hand, it was shown that in IL-1α transgenic mice, DMBA/TPA treatment resulted in reduced incidence of skin tumours, compared with control mice, possibly due to the rapid eradication of the arising malignant cells by innate effector cells that are activated by local IL-1α in the skin.[49]

NF-κB activation in pre-cancerous or inflammatory cells was recently shown to be an essential link between chronic inflammation and tumourigenesis.[50–53] Micro-environment-derived pro-inflammatory cytokines, i.e., TNF-α, activate NF-κB in target cells of experimental tumourigenesis systems; this rescues the cells from apoptosis and enables their progression in the malignant process. In inflammatory cells, NF-κB activation induces expression of pro-inflammatory cytokines that facilitate the growth of the pre-malignant cells and their development into overt tumours. There is an evident link between NF-κB and the pathway of IL-1 signalling that involves activation of NF-κB, which subsequently results in the expression of inflammatory genes, among them IL-1.

In our experimental system of 3-MCA carcinogenesis in IL-1 deficient mice, we have also observed that the 'IL-1 milieu',

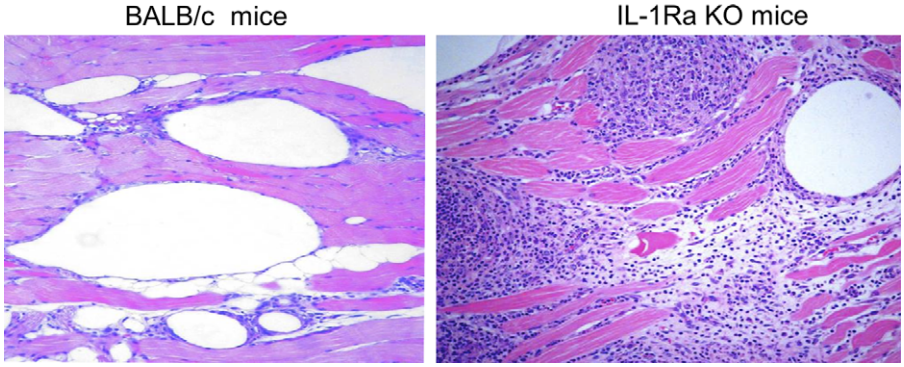

Fig. 3 – The inflammatory response at the site of carcinogen injection. Control and interleukin-1 receptor antagonist (IL-1Ra)$^{-/-}$ mice were injected with 3-methylcholantrene (3-MCA), as indicated in the legend to Fig. 2. Shown is the inflammatory response on d 10 after injection of the carcinogen. Haematoxylin and eosin (H&E) staining, × 20 magnification.

where malignant cells develop, affects their immunogenicity. Thus, transplantable fibrosarcoma cell lines obtained from IL-1α$^{-/-}$ mice, which failed to induce tumours in intact mice, generated tumours in sub-lethally irradiated mice, while 3-MCA-induced tumours from control mice. This indicates that the cells had not lost their invasiveness, as they are able to form tumours in immune-compromised mice. It is interesting that in spite of the similar tumour incidence in 3-MCA-treated IL-1α$^{-/-}$ mice, compared with control mice, the tumours were immunogenic in mice deficient in IL-1α. This is in accordance with recent reports on immunogenic tumour cells that arise in different immunodeficient 3-MCA-treated mice that lack critical components essential for the development of anti-tumour cell immunity (e.g., Rag-2$^{-/-}$, IL-12$^{-/-}$ and IFNγ$^{-/-}$ mice).[54–57] In control immune intact mice, these immunogenic variants are eradicated during tumour progression and low- or non-immunogenic tumours appear in such carcinogen-treated mice, in a process termed 'immuno-editing'. The process of immuno-editing in immunodeficient hosts allows the survival of malignant cell variants which are 'universally immunogenic' because they express surface adhesion or co-stimulatory molecules (e.g., ICAM-1 or 2, LFA-1 or 3, CD1d, VLA-4, B7, etc.). These adhesion molecules mediate high-affinity binding to immune effector cells, and serve as cell-associated co-stimulatory molecules, resulting in the activation of innate or specific immune surveillance mechanisms followed by the efficient eradication of the malignant cells. For example, it was shown that 3-MCA-induced immunogenic tumours that arise in IFNγ$^{-/-}$ mice all express CD1d, a ligand for NKT cells.[54–57] Most interesting, the vast majority (11/12) of fibrosarcoma cell lines, that had been established from 3-MCA-induced tumours in IL-1α$^{-/-}$ mice, express ICAM-1 that possibly contributes to their immunogenicity.

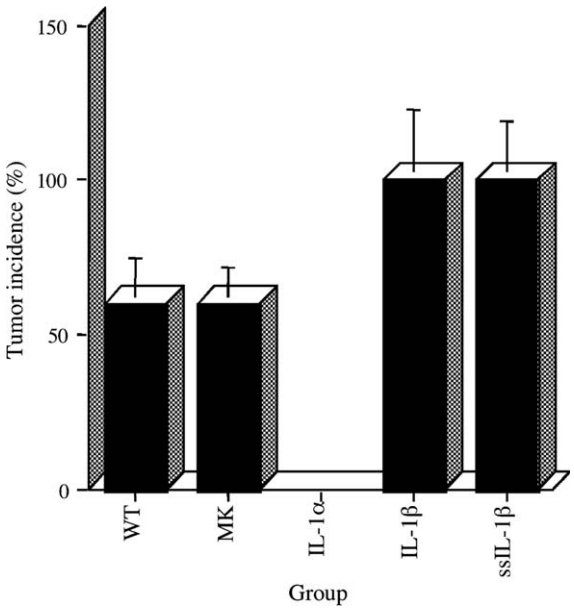

Fig. 4 – Invasiveness of fibrosarcoma cells overexpressing active forms of the IL-1 molecules. Wild-type (WT) cells were transfected with the precursor of IL-1α, the mature form of IL-1β and the mature form of IL-1β linked to a signal peptide (ssIL-1β). The parental wild-type cells and cells transfected with an empty vector (mock-transfected cells, MK) served as controls. Mice were injected with 2×10^5 tumour cells and the tumour incidence was assessed after 35 d. Results shown are the average from four experiment performed. Each experimental group consisted of 5–10 mice. The standard deviation (SD) of the mean is also shown.

4. Effects of IL-1 on tumour invasiveness, metastasis and tumour–host interactions

4.1. *Overexpression of IL-1α and IL-1β by malignant cells alters their tumourigenicity patterns*

We have assessed the role of tumour cell- and host-derived IL-1 on tumour invasiveness and metastasis. Initial studies involved the assignment of different functions to IL-1α and IL-1β in tumour invasiveness, using violent fibrosarcoma cells that had been transfected with active forms of the IL-1 molecules (see Fig. 4).

In fibrosarcoma cells which constitutively express pre IL-1α, as an aberration following transfection with oncogenes or with the cDNA of IL-1α, active cytokine is detected in the cytosol and on the cell membrane, but not in supernatants.[21–27,58] IL-1α-expressing cells lose their tumourigenicity due to the development of non-adaptive and specific immune responses, which lead to tumour regression. Histologically, an extensive mononuclear infiltrate, consisting of macrophages and lymphocytes was shown to invade initially developing tumours consisting of IL-1α-expressing fibrosarcoma cells; the tumour's mass was ultimately replaced by fibrotic tissue. In mice bearing IL-1α-transfected fibrosarcoma cells, efficient development of anti-tumour cell specific CTLs and a potent secretory response of Th1 cytokines, such as IFNγ and IL-2, were observed in the spleen. Non-specific effector mechanisms, such as NK cells and activated macrophages, were also activated by tumour cell-associated IL-1α. Infiltration of immune surveillance cells into the tumour and their local activation resulted in killing of the malignant cells and the regression of tumours, with the establishment of an immune memory which protects the mice from a challenge of the violent parental cells, as both types of cells share epitopes. The anti-tumour efficiency of therapeutic approaches based on tumour cell vaccines expressing IL-1α was also shown in mice bearing violent parental tumours. In addition, in vitro activation of malignant cells to transiently express cell-associated IL-1α by cytokines/immunomodulators was shown to be sufficient to induce tumour regression and led to the development of a protective immune memory.[26] Thus, membrane-associated IL-1α efficiently activates immune cells in a juxtacrine manner, via ligation of IL-1 receptors that are abundantly expressed on immune cells, and thus functions as a focused adjuvant to activate innate as well as specific anti-tumour immunity.

To assess effects of tumour cell-associated IL-1β on tumourigenicity patterns, we transfected violent fibrosarcoma cells with constructs bearing the cDNAs of the mature form of IL-1β and the mature form of IL-1β ligated to a signal sequence (ssIL-1β), to induce potent secretion of the mature form of IL-1β through the ER–Golgi pathway.[25,27] We found

that the IL-1β and ssIL-1β transfected fibrosarcoma tumours were more invasive than the violent parental cells or mock-transfected cells, exhibiting more rapid growth and earlier death of tumour-bearing mice (see Fig. 4). The invasiveness of the malignant cells grossly correlated with the amount of IL-1β that is secreted by them. In addition, only the ssIL-1β transfectants, which secrete relatively large levels of the cytokine, exhibited a metastatic potential, as manifested by the development of experimental metastases in the lungs after intravenous inoculation. Histologically, extensive mitosis and very few infiltrating leukocytes were observed in IL-1β and ssIL-1β tumour sites. No anti-tumour effector cells or cytokines that potentiate anti-tumour immunity (e.g., IFNγ and IL-2) could be detected in spleens of mice injected with IL-1β, ssIL-1β transfectants or in spleens from mice injected with the violent parental cells or mock-transfected cells. In contrast, effective anti-tumour cell immune responses were observed in mice injected with fibrosarcoma cells transfected with the precursor form of IL-1α, as indicated above.

Further studies have shown that in mice bearing tumours of IL-1β secreting cells, general anergy develops, seen by the suppression of Con A-induced T cell responses.[27] Suppression strongly correlates with the accumulation of $CD11b^{+}Gr\text{-}1^{+}$ immature myeloid cells in the peripheral blood and spleen concomitantly with the manifestation of other haematological alterations, such as splenomegaly, leukocytosis and anaemia. Immature $CD11b^{+}Gr\text{-}1^{+}$myeloid cells consist of cells committed to differentiate to granulocytes, macrophages, myeloid BM-derived dendritic cells as well as other early myeloid precursors (reviewed in Refs. 59–61). They were shown to suppress specific as well as non-specific T cell-mediated immune responses by mechanisms that are not completely understood. Such immature myeloid cells are an intrinsic part of the normal process of myelopoiesis and they are present in relatively small numbers in naive hosts. Their number significantly increases in tumour-bearing subjects and during infection. In our experimental system, systemic IL-1β levels of malignant cell origin induce these haematological alterations in tumour-bearing mice, with the hallmark of immune suppression and accumulation of immature $CD11b^{+}Gr\text{-}1^{+}$ myeloid cells in the spleen. This is part of the systemic inflammatory response induced by tumour cell-derived IL-1β, which includes leukocytosis, cachexia, liver necrosis and interstitial pneumonia (Song and colleagues). The effects of IL-1β on haemopoiesis are possibly mediated through effects on stromal cells in the marrow, to induce the production of cytokines that stimulate myelopoiesis or accelerated release of myeloid cells from the marrow to the periphery. Resection of large tumours of IL-1β-secreting cells restored immune reactivity and the haematological alterations within 7–10 d. Similarly, treatment of tumour-bearing mice with the physiological inhibitor of IL-1, the IL-1Ra, reduced tumour growth and attenuated the haematological alterations.

In spite of tumour-mediated suppression, resection of large tumours of IL-1β secreting cells, followed by a challenge with the violent parental cells induced resistance in mice; protection was not observed in mice bearing tumours of mock-transfected fibrosarcoma cells. Thus, in mice bearing tumours of IL-1β secreting cells, anti-tumour cell specific immunity is activated, due to the adjuvant-like effects of IL-1β, however, protective immunity is not manifested in tumour-bearing mice, possibly due to suppression of immune effector mechanisms.

In conclusion, secretable IL-1β acts in broad paracrine and endocrine manners; it is strongly pro-inflammatory, potentiating tumour angiogenesis and the production of a network of invasiveness-promoting molecules as well as tumour-mediated suppression.

4.2. *Effects of host-derived IL-1 on the invasiveness of transplantable tumours*

In order to assess the role of host-derived IL-1 in tumour invasiveness, we assessed the tumourigenicity of B16 melanoma cell in IL-$1\beta^{-/-}$ and IL-$1\alpha^{-/-}$ mice.[62] Our results have demonstrated that micro-environment-derived IL-1β, and to a much lesser extent IL-1α, is responsible for in vivo tumour angiogenesis and invasiveness of B16 melanoma cells (see Fig. 5A). In IL-$1\beta^{-/-}$mice, no local tumour development or lung experimental metastases were observed following intra-footpad or intravenous inoculation, respectively. In contrast, in control C57BL/6 mice, active tumour development was observed. In addition, in IL-$1\beta^{-/-}$ mice, no recruitment of a blood vessel network into Matrigel plugs containing B16 cells could be observed, while in control C57BL/6 mice, potent vascularisation of plugs was evident (see Fig. 5B).[62] To further substantiate the role of IL-1β in tumour angiogenesis, we have shown that addition of recombinant IL-1 into Matrigel plugs containing B16 cells in IL-$1\beta^{-/-}$ mice, partially restores the angiogenic response, while addition of IL-1Ra to B16-containing Matrigel plugs in control mice inhibited the ingrowth of the blood vessel network into the plugs. In IL-$1\alpha^{-/-}$ mice, local tumour development and induction of an angiogenic response in Matrigel plugs were observed, however, they were lower than those in control mice, but significantly higher than those in IL-$1\beta^{-/-}$ mice.[62]

The angiogenic response comprises a complex series of events that include the local degradation of the basement membrane, directional migration of the underlying endothelial cells, invasion of the surrounding stroma, endothelial cell proliferation, capillary tube morphogenesis, coalescence of small capillaries into larger vessels and vascular pruning and acquisition of peri-endothelial coating. Many of these phenomena are characteristic of inflammatory responses induced by IL-1β. As such, IL-1 may serve as a master cytokine upon which different angiogenic stimuli converge. Therefore, neutralisation of a single molecule, e.g., IL-1, may inhibit the generation of the cascade of downstream (effector) pro-inflammatory molecules with redundant functions in tumour angiogenesis. Indeed, continuous delivery systems of recombinant IL-1Ra or cells overexpressing IL-1Ra, encapsulated within alginate-poly (L-lysine)-alginate (APA) microspheres, reduced the tumour burden and inhibited tumour-mediated angiogenesis when implanted into tumour-bearing mice.[63]

The results provide pre-clinical support for the use of IL-1Ra as an adjunct therapy in combination with tumour resection and chemotherapy, to attenuate the growth and invasiveness of the tumour as well as to alleviate tumour-mediated suppression.

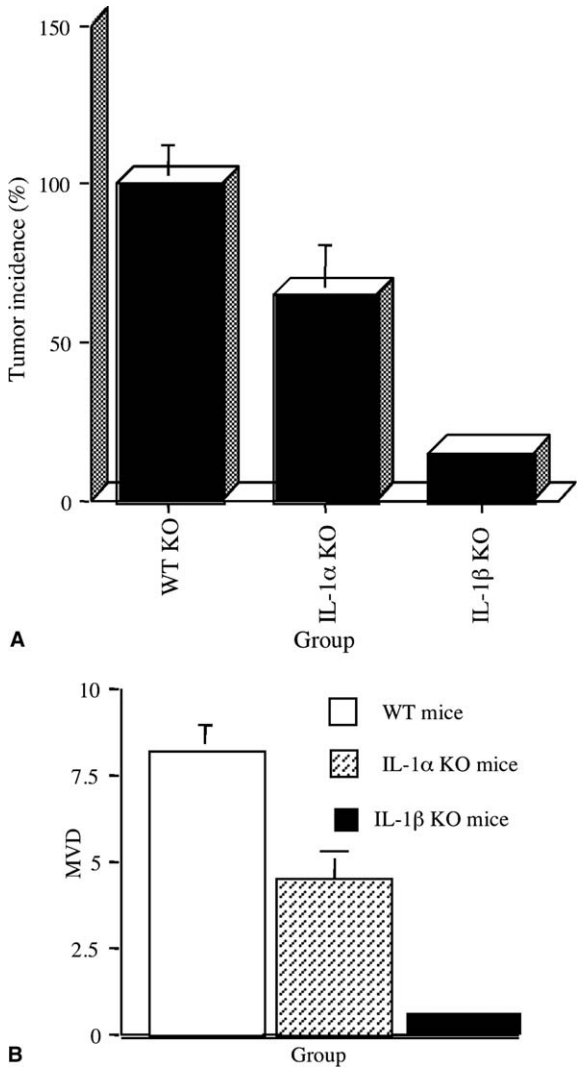

Fig. 5 – Invasiveness and angiogenesis patterns of B16 melanoma cells in IL-$1^{-/-}$ and control mice. (A) Invasiveness: tumour cells (2×10^5/mouse, intra-footpad) were injected into control (wild-type, WT), IL-$1\alpha^{-/-}$ or IL-$1\beta^{-/-}$ syngeneic C57BL/6 mice and tumour incidence was assessed on d 25. (B) Angiogenesis patterns: tumour cells (2×10^5/mouse) were mixed with Matrigel and injected subcutaneously. After 1 week, plugs were recovered and histological sections were prepared and stained with antibodies to Von Willerbrand's factor, a marker of endothelial cells. The mean vessel density (MVD) was calculated following counting positive blood vessels in six fields. Results shown are the average from four experiments. Each experimental group comprised 5–10 mice. The standard deviation (SD) of the mean is also shown.

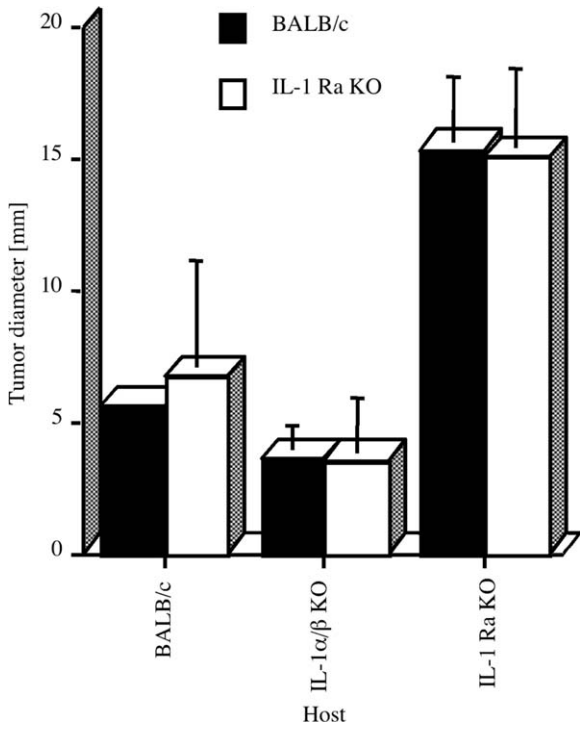

Fig. 6 – Tumourigenicity of 3-methylcholantrene (3-MCA)-derived fibrosarcoma cell lines, induced in control or interleukin-1 receptor antagonist (IL-1Ra)$^{-/-}$ BALB/c mice, in control and IL-$1^{-/-}$ mice. Cells from lines were injected (2×10^5/mouse, intra-footpad) into control, IL-$1\alpha/\beta^{-/-}$ (double IL-1 knockout (KO) mice) or IL-$1Ra^{-/-}$ BALB/c mice. The tumour size was assessed on d 30. Results shown are the average from four experiments. Each experimental group comprised 5–10 mice. The standard deviation (SD) of the mean is also shown.

4.3. *Tumour cell- and host-derived IL-1 are essential for tumour invasiveness*

Fibrosarcoma cell lines, isolated from 3-MCA-induced tumours in control and the IL-$1^{-/-}$mice, together with the IL-$1^{-/-}$ and control recipient mice, have been used in transplantation assays, to discriminate between the role of malignant cell- or host-derived IL-1 in determining the invasiveness potential of malignant cells. Tumour cells deficient in IL-1 genes are of reduced tumourigenicity in control mice and they express impaired patterns of angiogenesis in Matrigel plugs. The stimuli that turn on IL-1 production by malignant cells is as yet unknown. Deficiency in IL-1 expression by malignant cells may represent a failure to recruit cellular elements of the stroma or infiltrating of leukocytes, which are essential for tumour invasiveness. Cell lines that originated in 3-MCA-treated IL-$1Ra^{-/-}$ mice were more invasive and metastatic than similar cell lines obtained from control mice. This might be due to high unattenuated levels of IL-1 that are expressed in the malignant cells and facilitate their invasiveness. Similarly, deficiency in IL-1 expression or its unopposed expression by the host, reduced or increased the invasive potential of malignant cells, respectively. Thus, a 3-MCA-induced fibrosarcoma cell line that had developed in BALB/c mice manifested low invasiveness in IL-1 deficient mice, intermediate invasiveness in control mice and high invasiveness potential in IL-$1Ra^{-/-}$ mice (see Fig. 6).

5. Conclusion

Overexpression of IL-1β by the environment or the malignant cells potentiates carcinogenesis and the invasiveness of already existing malignant cells. At extreme levels of expression/

secretion of IL-1β in the tumour arena, general inflammatory manifestations, including haematological alterations and tumour-mediated suppression also occur. The role of cell-associated IL-1α, which is less diffusible in the micro-environment of the tumour, is less important in the process of carcinogenesis and tumour invasion. The feasibility of cancer management by the IL-1Ra has been demonstrated by us; it mainly acts to neutralise secretable IL-1β. IL-1Ra will be most probably effective after debulking primary tumours by surgery, chemotherapy or irradiation, to prevent tumour recurrence and metastasis, acting to limit tumour angiogenesis and invasiveness as well as to alleviate tumour-mediated suppression. It is feasible to use the IL-1Ra in cancer patients, as it is US Food and Drug Administration (FDA)-approved. It is currently used to reduce inflammation in patients with rheumatoid arthritis, with positive therapeutic results. On the other hand, malignant cells that do not secrete IL-1, but express cell-associated IL-1α, are of increased immunogenicity. As IL-1α is locally expressed and induces only limited inflammatory responses, its safe use in tumour cell vaccines overexpressing IL-1α can be envisioned, without risks that accompany local and systemic levels of IL-1β. Understanding the diverse functions of the IL-1 molecules in the tumour arena, in the distinct successive phases of the malignant process, will lead to development of novel therapeutic modalities based on intervention in IL-1 expression.

Conflict of interest statement

None declared.

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