Review

Tumour Biology: Tumour-associated Inflammation *versus* Antitumor Immunity

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Abstract. *The latest research results suggest that tumour-infiltrating leukocytes and the intra-tumoural cytokine environment play a central role in both the genesis and development of cancer. Over a hundred years ago, Virchow pointed out that numerous immune cells occur in the vicinity of practically all malignant tumours and that the structure of tumour tissue closely resembles the inflamed region of a non-healing wound. With the aid of the latest molecular and cell-biological methods, we are not only able today to closely characterise tumour cells and their immediate vicinity but also the other cell types present in tumour tissue, such as infiltrating immune cells, endothelial cells, connective tissue cells and others, both in terms of phenotype and function. In addition, there is growing understanding of the significance of the composition and functioning of endogenous messenger substances such as cytokines, chemokines and prostaglandins in healthy and malignantly altered tissues. From the immunological point of view, the main characteristics are dysregulated inflammatory conditions caused by the tumour cells themselves or by external factors, depending on the type of tumour event. It is evident that prolonged dysregulated inflammatory conditions favour not only carcinogenesis but also the local infiltration and metastasis of malignantly modified cells and counteract the development of efficient antitumor immunity. On the other hand, there are indications that through the polarisation of immunological reactions, the ability of immunological regulator and effector cells to induce efficient antitumor immunity can be modulated. Within the framework of this summary, the essential immunological aspects of tumour formation and tumour development known at present are presented and possible new therapeutic strategies are discussed.*

Abbreviations: APCs, Antigen-presenting cells; BCG, Bacillus Calmette-Guerin; BCL2, B-cell lymphoma 2; C(X)CR, chemokine receptor; CD, cluster of differentiation; COX2, cyclooxygenase 2; CTL, cytotoxic T lymphocytes; CTLA-4, cytotoxic T-lymphocyte antigen 4; DCs, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA, human leukocyte antigen; iNos, inducible nitrogen synthetase; INF-γ, interferon-gamma; IL, interleukin; LPS, lipopolysaccharide; MALT, mucosa-associated lymphoid tissue; MHC, major histocompatibility complex; MYC, myelocytomatosis viral oncogene homolog; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B-cells; NOD, nucleotide oligomerization domain; PCT1, cholinephosphate cytidylyltransferase; PRRs, pattern recognition receptors; RAS, Rat sarcoma; RET, rearranged during transfection; SIGIRR, single immunoglobulin interleukin 1 receptor related; STAT3, signal transducer and activator of transcription 3; TAMs, tumour-associated macrophages; TGF, transforming growth factor; T_H-response, T-helper cell response; TLRs, toll-like receptors; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VHL, von Hippel–Lindau.

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Over a hundred years ago, renowned scientists had already drawn attention to a correlation between malignant and inflammatory changes in tissue (1). This was based on new histological methods by means of which numerous immune cells could be identified in the vicinity of almost all tumours. At that time, however, the methods were neither capable of identifying specific phenotypical or functional characteristics

of the immune cells infiltrating the tumour, nor could they identify the particular cytokine environment. From today's point of view, it is therefore not surprising that, following the triumphant development of molecular biology and the concomitant focus on genetic modifications of degenerated cells, nonspecific aspects such as the cytokine environment, tumour bystander cells or dysregulated inflammation have received hardly any attention. On the basis of the latest molecular and cell-biological examination methods, it is becoming increasingly possible to verify even more complex immunobiological interrelations in normal and degenerate tissues (2). Thus, epidemiological studies indicating that immunological reaction conditions such as dysregulated chronic inflammation do in fact have a close, in part even a causal, relationship to the formation and development of tumours, are confirmed (3-5). So it can be estimated that in 20% of all people dying from cancer, the tumours were directly or indirectly related to infections and/or uncontrollable inflammatory conditions. In addition, numerous studies indicate that chronically dysregulated inflammation significantly increases the risk of suffering from cancer. In apparent contradiction to this are studies which show that, under certain circumstances, inflammatory reactions may also lead to the shrinkage or even the disappearance of tumours (6). Although such phenomena are, indeed, of great significance from the clinical point of view, they are little understood at present. It is against this background that we examine the latest immuno- and tumourbiological research findings and discuss them with regard to the development of future antitumor therapy strategies.

Nonspecific Inflammation and Carcinogenesis

Both immunological regulator and effector cells as well as the corresponding cytokines, chemokines and prostaglandins, can be identified within the framework of chronic inflammation reactions in almost all malignant tissue changes (1-4). Acting as inflammatory mediators, they not only influence the nature of the local immune response but also significantly influence the intratumour environment and thus the development of the tumour. In many ways the wide range of mediators involved in tumour development such as vascular endothelial growth factor (VEGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factor beta (TGF-β), interleukins *etc*. are a reflection of the regeneration and wound healing processes (5). Correspondingly, within the framework of malignant tissue changes, activated connective tissue cells, endothelial cells, branches of nerve cells and such like are found in addition to tumour cells (2). One common feature of these cells is that they do not develop normally in the tumour tissue. Thus malformed blood and lymph vessels, degenerated nerve endings or numerous necrotic cells are to

be found. As Virchow already described over a hundred years ago, in terms of tissue morphology, tumour tissue resembles a chronically infected non-healing wound (5). It is interesting that numerous intracellular signal transduction pathways activated by pro-inflammatory mediators lead to the activation of oncogenes, such as *RAS, MYC* and *RET*. Correspondingly, specific inhibition of pro-inflammatory mediators, such as tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) also leads to a reduction in tumour development and the inhibition of central transcription factors associated with inflammation processes such as nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) or signal transducer and activator of transcription 3 (STAT3) (7-10).

Current studies show that NF-κB in particular plays a fundamental role in the formation and development of malignant tissue changes caused by inflammation (11). As a ubiquitous central transcription factor, NF-κB plays a role both in the transformation of tissue cells to cancer cells, as well as in the regulation of the activation of immune cells (11, 12). If, for example, immunological regulator or effector cells are confronted with microorganisms, this leads to the stimulation of pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors and cytosolic helicases. The activation of the innate immune system *via* TLR has been most closely studied so far. Depending on the receptor, they lead particularly *via* the MyD88 or TRIF signal pathways to activation of the NF-κB transcription factor (13, 14). The stimulation of immune cells by inflammatory cytokines such as interferon, TNF-α or IL-1β also leads ultimately to the activation of NF-κB and thereby to nonspecific inflammatory reactions. In tumour cells, NF-κB is activated in particular by genetic mutation and gene amplifications (15). Here too, the continued activation of NF-κB leads to the increased expression of genes which encode inflammation-promoting cytokines, adhesion molecules, angiogenic factors, cyclooxygenase 2 (COX2), inducible nitrogen synthetase (iNos) *etc*. Furthermore, through the increased expression of anti-apoptotic genes such as *BCL2*, NF-κB activation promotes the survival of cancer cells (16). Interestingly, there are growing indications of an interaction between the NF-κB and HIF1α systems, synonymous with a connection of the innate immune system with the tissue response to hypoxia (17). The NF-κB signal pathway is strictly controlled at several levels. One example is the single immunoglobulin interleukin 1 receptor related (SIGIRR). This member of the IL-1 receptor family inhibits signalling by TLRs and the IL-1 receptor and is strongly expressed in the cells of the intestinal mucosa. In animal studies, genetically caused deficits in the expression of SIGIRR increase sensitivity to inflammation and carcinogenic changes in the intestinal epithelium (18). Comparable with NF-κB, STAT3 is a key

molecule for numerous oncogenic signal transduction pathways (19). The activation of this transcription factor through mutation or pro-inflammatory mediators in tumour cells leads to the inhibition of apoptosis and inhibition of an effective immune response by suppression of the maturation of antigen-presenting cells (20, 21).

Nonspecific Inflammatory Reactions and the Spread of Tumours

Most studies on the influence of nonspecific inflammatory reactions on carcinogenesis are concerned with early stages of tumour development. However, pro-inflammatory mediators such as cytokines, chemokines and their receptors, metalloproteinases and others also play a significant role in the infiltration of tumour cells into healthy tissue and the formation of metastases in other organs (22). For example, the chemokine receptor CXCR4 and its ligand CXCL12 play a key role in cell mobility in both diseased as well as healthy tissue (23). CXCR4 is not only expressed more frequently by malignant cells, the level of expression also correlates with the level of lymphogenic metastasis in various tumour entities (24-26). Other chemokine receptors such as CX3CR1, CCR1, CCR7, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR5 and CXCR7 are also expressed by malignant cells and are not infrequently involved in the migration of tumour cells to healthy tissue (27-34). Thus the expression of CCR7 correlates with the formation of metastases in lymph nodes and CCR9 with the metastases of melanoma cells in the small intestine. Interestingly, melanoma cells in particular frequently express chemokine receptors, a factor which may be responsible for the increased tendency for the formation of distant metastases. However, how do malignant cells acquire the ability to express chemokine receptors? Some mechanisms, such as autocrine or paracrine extracellular signals as well as genetic modifications have been described. For example, mutations in the tumour suppressor Von Hippel–Lindau (VHL) lead to an increased expression of CXCR4. In addition, the ability of tumour cells to infiltrate tissue increases in the presence of cytokines such as TNF-α, IL-1 and IL-6, possibly as the result of an increased expression of chemokine receptors (35). Thus the autocrine formation of TNF-α leads to an increased expression of CXCR4 in ovarian carcinoma cells (36). Correspondingly, a reduction in CXCR4 expression and thereby not only an inhibition of angiogenesis but also a reduced spread of tumours in the peritoneal region as well as in more distant organs can be achieved by the inhibition of TNF-α. Furthermore, TNF-α promotes the epithelial-mesenchymal transformation of intestinal cancer cells (37). Irrespective of these specific mechanisms, the ability of tumour cells to increase the expression of chemokine receptors seems to be a general feature of malignant cells

with an epithelial or mesenchymal origin in early and, above all, in advanced stages of tumours.

In addition to inflammatory mediators, immune cells also play a significant role in the progression of tumourous diseases. Thus, tumour-associated macrophages (TAMs), for example, are not only obligatory partners in the migration of cancer cells and their invasion into the surrounding tissue but also in the formation of metastases in other organs (38-40). Moreover, carcinogenic cells of the breast in a genetically modified macrophage-deficient mouse model were not capable of metastasising in contrast to those of a wild mouse (39). In addition to a direct interaction of macrophages and tumour cells, the paracrine formation of various growth factors seems to decisively promote metastasis (41). It is also interesting in this context that tumour cells co-cultivated with macrophages develop an increasingly metastatic phenotype, comparable with the activation of the NF-κB pathway or activation *via* TNF-α (41).

The Causes of Nonspecific Tumour-associated Inflammatory Conditions

Nonspecific inflammatory conditions in malignantly altered tissue may have many causes (Figure 1). With regard to their pathogenesis, they are initiated by the tumour cells themselves intrinsically through genetic alterations or extrinsically through external factors, which stimulate tumour cells to release increasing amounts of pro-inflammatory mediators. The latest studies also show that tumour-independent chronic inflammatory conditions may promote the malignant degeneration of body (stem) cells.

The intrinsic mode of inflammation induction describes the initiation of a tumour-associated inflammatory reaction as a result of intracellular and, in particular, genetic alterations in the tumour cells themselves (43). In numerous pre-clinical tumour models, it was demonstrated that tumour cells can initiate or maintain an inflammatory environment in their vicinity without any identifiable involvement of specific external causes such as bacteria, noxa *etc*. In this context, the papillary thyroid carcinoma is a landmark clinical model. The early stage of carcinogenesis is represented by the chromosomal rearrangement of RET/PCT1 and thus activation of the corresponding oncogene (44). In a corresponding *in vitro* model with freshly isolated human thyrocytes, activation of RET leads to an increased expression of numerous cytokines, chemokines, chemokine receptors, metalloproteinases or adhesion molecules associated with inflammation processes. RET-associated inflammatory mediators have in the meantime been identified in tissue biopsies from widely differing tumours as well as in benign diseases (45). These studies show that early genetic changes which lead to the formation of carcinoma also intrinsically promote local inflammatory reactions (44-45).
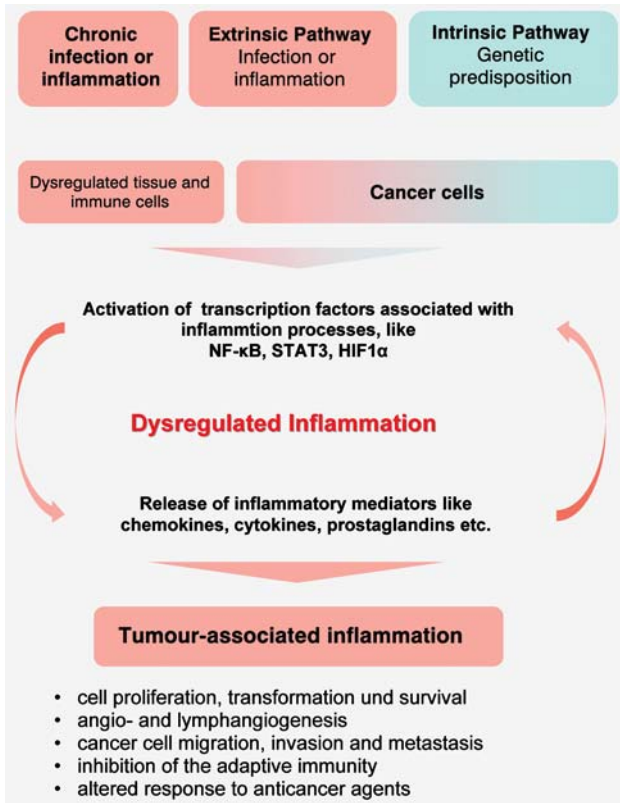
Figure 1. *Tumour-associated inflammation. The term tumour-associated inflammation describes chronic dysregulated inflammation processes of different origins. Located in the tissue this kind of inflammation promotes the genesis and spread of cancer. It can be caused by transformed cancer cells as well as by dysregulated tissue or immune cells producing inflammatory mediators including cytokines, chemokines, cyclooxygenase 2 (COX2) and prostaglandins thereby generating a tumour-promoting inflammatory microenvironment.*

Figure 2. T_H1-*Response versus intracellular infection. Inverse proportionality of T_H1-response and the development of intracellular infection with viruses, mycobacteria or parasites.*

angiogenesis as well as tumour growth (49). This study and further studies show that important oncogenes such as RET, RAS and myc significantly influence the inflammatory environment as well as the structure of the malignantly modified tissue, *via* an intrinsic pathway.

With regard to carcinogenesis, previous pre-clinical studies have not been sufficiently able to explain the complex interplay of genetically and extrinsically induced inflammatory incidences. This interplay is probably the basis for the formation of the majority of clinically relevant, spontaneously occurring tumours. Exemplary of this could be the pathogenesis of pancreatic ductal carcinoma, in which both pancreatitis and KRAS mutations appear to play key roles. In a corresponding mouse model, adult mice were resistant to mutated KRAS-induced pancreatic ductal cancer (46). For carcinogenesis, both are apparently necessary, a weak chronic inflammation of the pancreatic duct as well as the mutated KRAS oncogene. Even more evident is the influence of inflammation in tumour disease, which frequently occurs as a result of chronic inflammation of the intestine such as in colitis ulcerosa, Crohn's disease (50), or following chronic infections with *Helicobacter pylori* such as in stomach cancer or mucosa-associated lymphoid tissue (MALT) lymphoma (51). A prolonged activation of immunological regulator and effector cells is evident in this. If the immune system does not succeed in eliminating the specific inflammation stimulus (bacterial constituent, contaminants, noxi *etc.*) the inflammatory reaction becomes chronic. In animal models, it has been clearly demonstrated that, for example, a prolonged helicobacter infection can trigger chronically-dysregulated inflammatory reactions. If the inflammation lasts several months, it promotes the formation of adenocarcinoma (52). If the local inflammatory reaction is prevented, for example by cyclooxygenase inhibitors or the bacteria are destroyed using antibiotics, the formation of stomach carcinoma can be significantly restricted (53). The fact that these studies are also relevant for clinical applications is shown by clinical studies on the incidence of gastric carcinomas (54, 55) or on the development of adenocarcinomas in patients

A further example is that of the members of the RAS family. They are amongst the most frequently mutated oncogenes in human tumours. Activated oncogene components of the RAS-RAF signal transduction pathway induce the production of numerous pro-inflammatory mediators (46, 47). A further oncogene, myc, codes for a transcription factor, which is also over-expressed in numerous human tumours. The deregulated expression of this oncogene initiates and influences central aspects of the tumour phenotype. Thus, myc supports autonomous cell growth, influences cellular microstructure, angiogenesis and the microenvironment of the tumour tissue (48). Furthermore, myc induces the formation of numerous chemokines, which for example promote the migration of mast cells into malignantly altered tissue. Through the formation of numerous pro-inflammatory mediators, mast cells, on the other hand, are capable of promoting
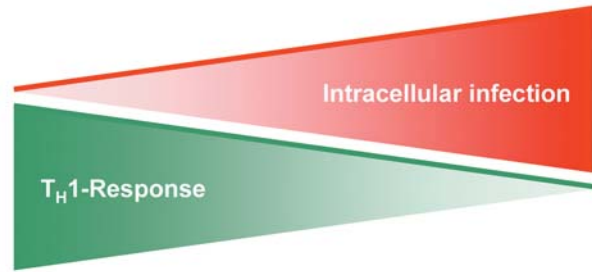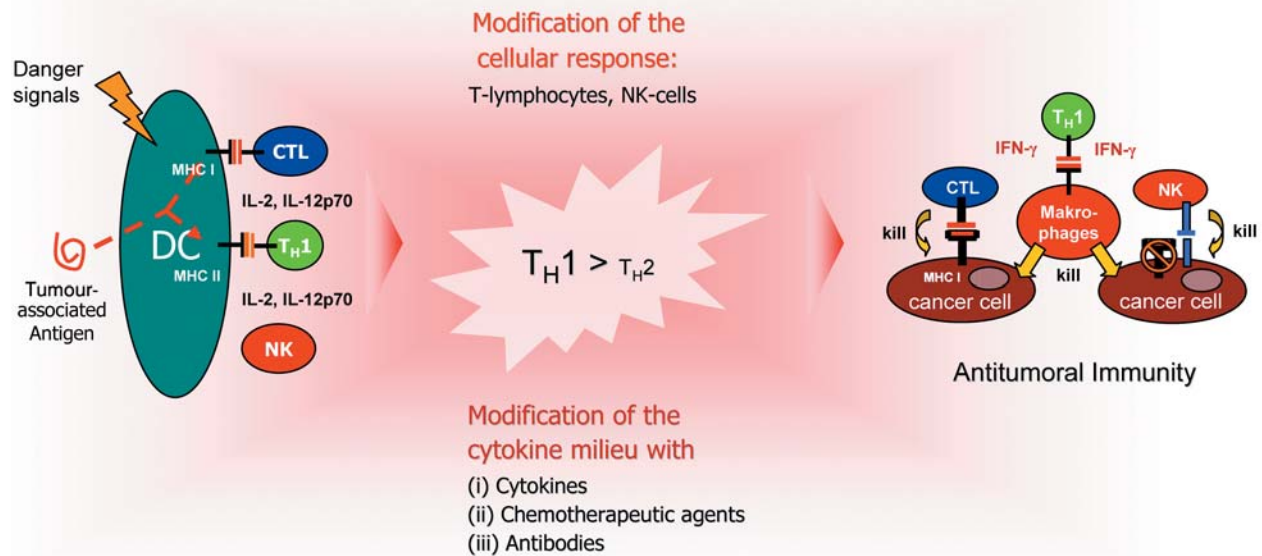
Figure 3. *Concept of the induction of an effective antitumor immunotherapy with antigen-loaded T_H1-polarised dendritic cells. To induce an effective antitumoral immune response and to overcome the tumour-associated immune tolerance both an effective cellular response against cancer cells as well as the modification of the cytokine milieu is needed. Therefore, monocytes were isolated from the peripheral blood of the patients and differentiated under the influence of IL-4 and GM-CSF to immature MoDCs in vitro. Loaded with tumour-associated antigens und matured with defined danger signals they present antigen-peptides by MHC I + II and produce especially IL-12p70 and IL-2 inducing both an antigen-specific cytotoxic (CTL) and helper T-cell (T_H) response as well as a T_H1-polarised cytokine milieu. Latter supports the activation of further immune cells like NK-cells, macrophages etc. and can be inforced by the additional application of (i) cytokines e.g. IFN-γ or IL-12p70, (ii) chemotherapeutic agents e.g. cyclophosphamid or fludarabin or (iii) antibodies against IL-4, IL-10 or CTL4.*

with corresponding genetic predisposition (56). In the latter, the recurrence of adenoma could even be prevented by the regularly administration of COX2 inhibitors (56, 57). Also interesting in this context are studies on carcinogenesis in mice with bone-marrow transplants. These indicate that the adenocarcinoma cells, characteristic of gastric carcinomas, are primarily recruited from mesenchymal stem cells (58). In these studies, female mice were transplanted with the bone-marrow of syngenic males after whole-body irradiation and then infected with helicobacter. In the gastric adenocarcinomas which developed after approximately 8-12 months, up to 70% Y-chromosome carrying donor cells were found. Further studies showed that these cancer cells derived from mesenchymal bone-marrow stem cells (59). On the basis of this and comparable studies, the following immunobiological scenario is feasible. Infection with helicobacter leads to the recruitment of immunological regulator and effector cells such as macrophages, dendritic cells, T and B lymphocytes and granulocytes. These are normally capable of eradicating the bacteria completely. If this does not occur, as in the mouse model, chronic infections, and thus chronic immunological defence reactions, are the result. As in a chronic, non-healing wound, increased numbers of immune mediators are produced, leading to further immune cells migrating into the region of inflammation. In addition, in the course of regeneration of the damaged gastric mucosa, local and mobile,

in particular mesenchymal, stem cells are activated or attracted. Finally these meet dysregulated inflammation and tissue structures, which do not allow regular development or wound healing. Should this process continue, it is easy to explain why, in the corresponding mouse models, the developing gastric adenocarcinoma cells primarily are recruited from tissue stem cells. Such models can also explain, for example, why chronic inflammations of the pancreas, liver, prostate or bladder significantly increase the risk for the incidence of carcinomas in the corresponding tissue region (60-64) and why chronic inflammatory changes in tumour tissue increase the risk of metastasis (65-68). It also becomes more plausible why tumours modified by inflammation exhibit an increased tendency for disseminated spread and why tumours can suddenly spread or shrink or even regress completely as a result of inflammatory or septic processes (69). Correspondingly, anti-inflammatory substances such as chemokine receptors, cytokine receptor antagonists and COX inhibitors are capable of having a beneficial influence not only on the development of tumour and metastasis, but also on the incidence and mortality of numerous tumour entities (70-73). In numerous phase I/II studies, the use of antagonists for IL-6, IL-6 receptor, CCL2, CCR4 and CXCR4 in the therapy of epithelial end haematological neoplasia is under examination at present. Initial clinical studies (phase I/II) with TNF-α antagonist in patients with advanced

tumour disease have lead in some cases to stabilisation, and in patients with renal cell carcinoma even to partial tumour regression (74-77). The structural analogue to thalidomide, lenalidomide, in combination with dexamethasone has also proven to be effective in the therapy of advanced myeloma. Like thalidomide, lenalidomide prevents the production of a whole range of inflammation-promoting cytokines (78, 79).

Tumour-infiltrating Leukocytes (TILs) and Cytokine Environment

Leukocytes which have invaded malignantly modified tissue can be identified in almost all tumours. Their composition, number and distribution vary not only between individual but also between tumour sites in the same organism. They are composed mainly of immunological regulator and effector cells such as TAMs, dendritic cells (DCs), mast cells and lymphocytes. There are many indications that each of these immune cell types which originate from bone-marrow can be involved in carcinogenesis, local tissue infiltration by tumour cells, as well as in the migration of cancer cells to other organs (41, 80-85). A number of pre-clinical studies show that antigen-presenting cells (APCs) such as tumour-associated macrophages or myeloid DCs as well as mast cells form the basis by which leukocyte infiltrates can benefit or restrict the progress of tumour development. The prevalent cytokine environment appears to play a significant role in this. However, the oncogenetic interplay between these types of cells and their respective significance within the framework of tumour development are only beginning to be understood.

A possible breakthrough, which is also therapeutically relevant, may arise from the molecular and cell biological evidence that intratumoral APCs such as macrophages and DCs frequently show a T-helper 2 (T_H2)-polarisation in murine and human tumours (86, 87). Contrary to T_H1-polarised APCs, characterised in particular by the production of high amounts of functional IL-12p70 and the induction of an antigen-specific cytotoxic immunity (88), T_H2-polarised APCs produce cytokines such as IL-10, IL-5 and TGF-β. By preventing the induction of an antigen-specific immune response, the latter support immunological tolerance mechanisms (also to tumour antigens) and thereby inhibit the formation of an adaptive antitumor immunity (89). Tolerance is also reinforced by regulatory T-cells (Treg) or mast cells, as well as by inflammation mediators intrinsically formed by tumour cells (82, 83, 90).

The significance of polarised immune responses or inflammatory conditions is best demonstrated in studies on the spread of intracellular infectious agents (Figure 2). It has long been known that viruses, mycobacteria (tuberculosis or leprosy agents) or parasites such as Leishmania can spread particularly freely in the body if the host is unable to form sufficient T_H1 response (91, 92). This in turn is based on T_H-

cells, which differentiate from T_H0 to T_H1 cells on contact with T_H1-polarised DCs. T_H1 cells, together with the key molecule interferon gamma (IFN-γ), produce a T_H1-cytokine pattern, including cytokines such as IL-2, TNF-α and GM-CSF. T_H1 cytokines, on their part, support the T_H1-polarisation of APCs, in particular of DCs, and thereby strengthen the induction of efficient antigen-specific cytotoxic immunity (93). At the same time IFN-γ, in particular, restricts the differentiation of T_H0 cells to T_H2 cells and thus prevents the formation of immunological tolerance (94). In contrast, the cytokines formed by T_H2 cells, such as IL-10, IL-4, IL-5 etc., restrict the development of a T_H1 response (94).

In this context, it seems particularly significant that the latest clinical studies into adaptive immunity in various tumours such as colorectal carcinoma, ovarian carcinoma, carcinoma of the bladder and glioblastoma not only indicate a positive correlation between tumour infiltrating lymphocytes and the survival of the affected patients (95-99) but also, as in colorectal carcinoma in particular, indicate the prognostic significance of an intra-tumour T_H1 cytokine environment (99). Thus, a leading study by Galon et al., examining 415 patients with colorectal carcinoma, showed that the type, density and precise localisation of CD3- or CD8-positive lymphocytes in the tumour tissue provide a better prognostic indication than the usual clinical UICC-TNM classification which focuses on tumour spread. Furthermore, using corresponding expression analyses (real-time PCR, tissue microarray), evidence was found in 75 patients with colorectal carcinoma for a significant negative correlation between the expression of genes associated with a T_H1 response and the risk of relapse after the complete operative removal of the tumour (99). In contrast to this, an immune-suppressive or T_H2-polarised tissue environment correlated positively with the recurrence of the tumour disease. The results of this work thus support the hypothesis that it is not solely a nonspecific inflammatory reaction but rather above all the T_H1/T_H2-polarisation of the immune response which governs the ability to develop an efficient adaptive antitumor immunity. However, in this context it must be pointed out that, particularly for advanced tumour diseases, no stringent clinical studies have been carried out to date with respect to the link between a functional polarisation of the tumour environment and the progress or prognosis of tumour disease.

Antitumor Immunity

Therapies against cancer which aim at a sustained strengthening of the immune system are not new. At the end of the nineteenth century, William Coley recorded that some of his cancer patients who developed serious postoperative infections in the region of the tumour exhibited spontaneous and sustained tumour regression (100). As a result he developed the Coley toxin, a filtrate of cultures of

Streptococcus pyogenes and *Serratia marcescens*, which was injected directly into the tumour or the tissue surrounding the tumour. Although both the technique used and the results obtained were the subject of controversial discussions at that time, Coley documented cases with long-lasting remission in tumours which even today can only be healed in exceptional cases. Even before Coley, there were convincing indications of regression in advanced tumours following bacterial infection. Animal models in fact show that, following the activation of innate immune responses by bacteria or their surface molecules such as lipopolysaccharides and lipopeptides, tumours regress or disappear completely. Of central significance to immune and effector cells here are TLRs (101). This is even more surprising given that cellular activation *via* TLRs or the TLR adapter protein MyD88 is shown to play a key role in inflammation-associated carcinogenesis, tumour development and metastasis (68, 102). Here, the nature or polarisation of the antitumor response seems to play a significant role in antitumor effectiveness as well. Thus activation *via* TLRs by means of TLR agonists not only leads to nonspecific inflammatory reactions, but also to an antigen-specific adaptive immune response. For example, if DCs located in the tissue recognise pathogens *via* TLRs, they mature into efficient APCs and migrate to the regional lymph nodes. There, they induce an adaptive immune response to antigen epitopes of the pathogen in question (93). Depending in particular on the activation status of the DCs, the prevailing cytokine environment and the TLR agonist, polarisation of the immune response into T_H1 or T_H2 direction will occur and thus either the formation of an efficient antigen-specific cytotoxic T-cell response or the induction of an antigen-specific immunological tolerance (103).

In clinical terms, the concept of the generation of antitumor immunity with the aid of bacterial agents or *via* TLR agonists is only employed in a standard form in relatively few tumours. Examples are local cancer of the bladder in which a strong local inflammatory reaction is induced using mycobacteria (*Mycobacterium bovis bacillus* Calmette-Guerin, BCG) and basal cell carcinoma (104, 105). In the latter, treatment with the active agent, Imiquimod, gives relatively high rates of response. Imiquimod is a TLR7 agonist and, like other TLR agonists, leads together with IFN-γ to a T_H1-polarised inflammatory reaction (106, 107). Here too, the decisive factor seems to be the dominant cytokine environment prevailing prior to or during activation of the effector and regulator cells. In our own studies, we were able to show the central significance of interferons in the activation and polarisation of DCs with differing TLR agonists both *in vitro* and in animal models (108, 109). On the basis of this and within the framework of a clinical vaccination study in 12 patients with progressive hormone-refractory prostate carcinoma using autologous PSA peptide-

loaded DCs (MoDCs) generated from monocytes of peripheral blood, we studied the influence of systematic pre-treatment with IFN-γ on the induction of antitumor immunity (110). Although we were not able to observe any prolonged significant activation of PSA peptide-specific effector cells in any of the cases, in four of the patients treated in this way, a stabilisation of the illness occurred, and in two patients there was a clear regression of the tumour. Above all, it was significant that only those patients with relatively low tumour incidence and largely normal immune parameters showed a positive clinical response. In the subsequent clinical investigations, we also optimized the T_H1 polarisation of the PSA peptide-charged MoDCs used in the vaccination with a cytokine maturation protocol specially designed for clinical application (111). MoDCs matured in this manner are characterized in particular by the fact that they secrete bioactive IL-12p70. In animal models, T_H1-polarised DCs improve antitumoral immunity by activating NK cells and antigen-specific cytotoxic T-cells (81, 103). Initial clinical studies with improved T_H1-polarised DCs and IFN-γ on patients with advanced prostate carcinomas showed a significantly improved effectiveness of this vaccination approach, even in patients with far-advanced tumours. It will be interesting to see whether we will succeed in sustainably improving the induction of antitumor immunity by further optimising the T_H1 response, for example with the help of (i) more efficiently T_H1-polarised DCs, (ii) chemotherapeutic agents such as cyclophosphamide or fludarabin, or (iii) antibodies against IL-4, IL-10 and CTLA-4 (Figure 3).

Conclusion

The latest research results clearly show that dysregulated immunological regulator and effector mechanisms are involved in the genesis and spread of tumours. However, it remains unclear whether the resulting dysregulated inflammation and the tumour-infiltrating leukocytes involved in the inflammation are sufficient for the development of cancer and what role exogenous or endogenous carcinogenic agents play in this context. Furthermore, clarification is needed of which inflammation mediators and immune cells, in which kind of tumour event benefit or even prevent the development and spread of malignantly altered cells. In this context, further studies of cell biology and molecular biological mechanisms forming the basis of tumour-associated inflammation would prove highly useful. Particular attention should hereby be paid to the T_H1/T_H2 polarisation of the cytokine milieu in malignantly altered tissue as well as in healthy tissue.

With regard to the development of new therapeutic strategies, a decisive factor will be the transfer of knowledge about the inflammatory conditions linked to malignant cell modifications into concrete therapeutic measures. In the final

analysis, tumour cells are difficult targets acquiring resistance during their development. Therefore, in terms of therapy, it could be highly beneficial not only to directly attack malignant cells, but also to modify the corresponding inflammatory environment in order to favour the induction of sustainable antitumor immunity. The potential of using new approaches in therapy based on scientific findings in order to directly switch off tumour cells but also to influence the tumour environment such that the body's own innate defence mechanisms take effect, opens up new and fascinating perspectives for the sustainable therapy of tumourous diseases.

Acknowledgements

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