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TNF: A master switch for inflammation to cancer

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1. ABSTRACT

Chronic inflammation has long been associated with the development of cancer, ever since Rudolf Virchow's first observation that leukocytes were present in neoplastic tissue more than 130 years ago. Recent evidences have reignited the interest of cancer researchers in the exciting concept of an association between chronic inflammation and cancer. Tumor necrosis factor alpha (TNF-alpha), initially discovered as a result of its antitumor activity, has now been shown to be one of the major mediators of inflammation. Induced by a wide range of pathogenic stimuli, TNF-alpha induces other inflammatory mediators and proteases that orchestrate inflammatory responses. TNF-alpha is also produced by tumors and can act as an endogenous tumor promoter. The role of TNF-alpha has been linked to all steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis. How TNF-alpha acts as a masterswitch in establishing an intricate link between inflammation and cancer is the focus of this review.

2. INTRODUCTION

Tumor necrosis factor (TNF)-alpha and TNF-beta, produced primarily by monocytes and lymphocytes, respectively, were first isolated in 1984, as cytokines that kill tumor cells in culture and induce tumor regression *in vivo* (1). Intravenous administration of TNF to cancer patients produced numerous toxic reactions including fever (2). In animal studies, TNF has been shown to mediate endotoxin-mediated septic shock (3). Other reports have indicated that dysregulation of TNF synthesis mediates a wide variety of diseases including cancers and various inflammatory conditions such as rheumatoid arthritis, inflammatory bowl disease (also called Crohn's disease).

3. TNF-ALPHA CELL-SIGNALING

TNF-alpha is a transmembrane protein with a molecular mass of 26 kDa that was originally found to be expressed in macrophages and has now been found to be expressed by a wide variety of cells. In response to various stimuli, TNF-alpha is secreted by the cells as a 17 kDa

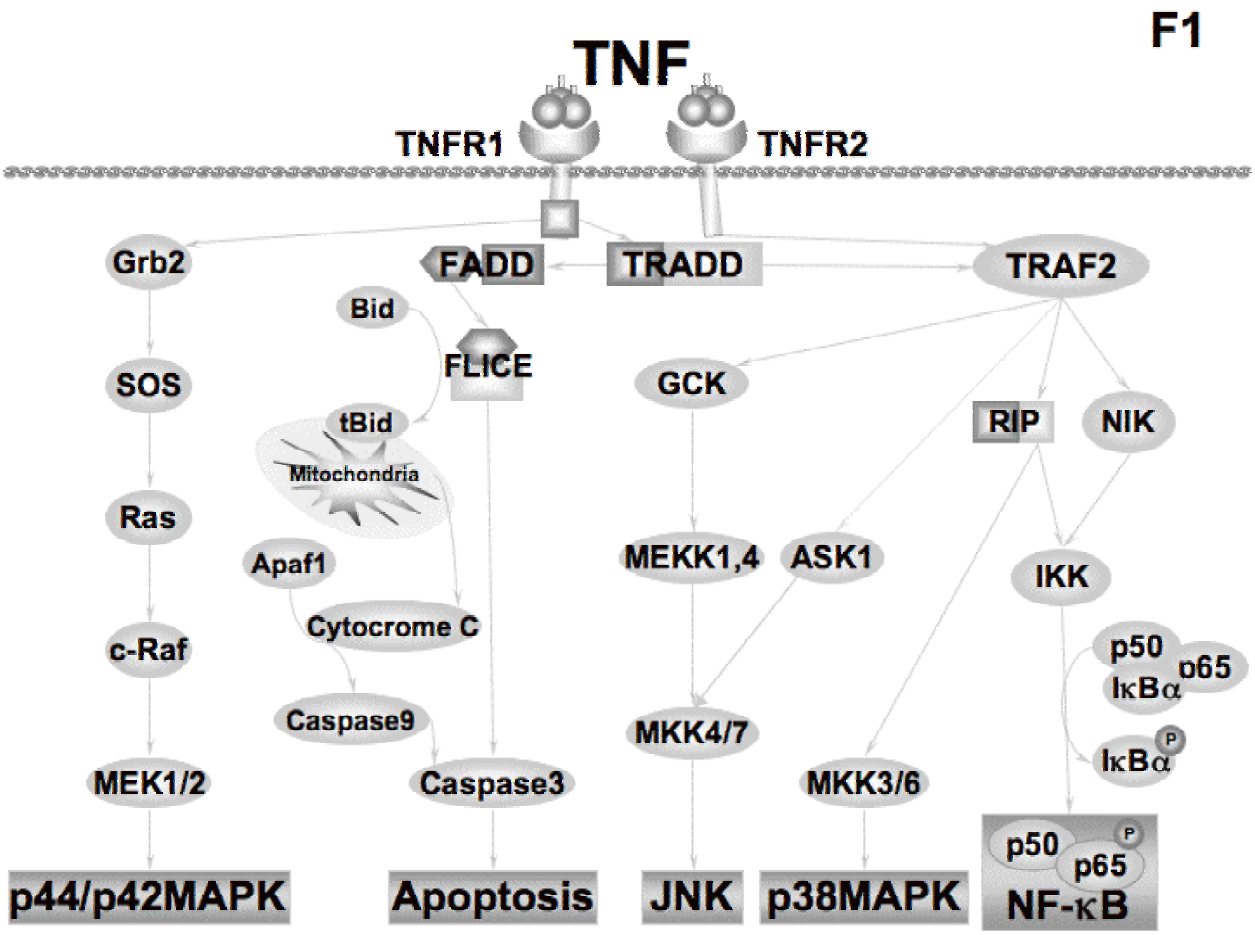

Figure 1. TNF signaling pathway.

protein through a highly regulated process that involves an enzyme TNF-alpha-activating converting enzyme (TACE) (4).

TNF-alpha mediates its effects through two different receptors: TNF-alpha receptor I (also called p55 or p60) and TNF-alpha receptor II (also called p75 or p80). While TNF-alpha receptor I is expressed on all cell types in the body, TNF-alpha receptor II is expressed selectively on endothelial cells and on cells of the immune system (4, 5). TNF-alpha binds to two receptors with comparable affinity. Why there are two different receptors for TNF-alpha is incompletely understood. Evidence related to differential signaling (5), ligand passing (6), binding to soluble TNF-alpha *vs* transmembrane TNF-alpha (7) has been presented.

The cytoplasmic domain of the TNF-alpha receptor I has a death domain, which has been shown to sequentially recruit TNF-alpha receptor-associated death domain (TRADD), Fas-associated death domain (FADD), and FADD-like ICE (FLICE) (also called caspase-8), leading to caspase-3 activation, which in turn induces apoptosis by inducing degradation of multiple proteins (8). TRADD also recruits TNF receptor-associated factor (TRAF2), which through receptor-interacting protein (RIP) activates IkappaBalpha kinase (IKK) leading to IkappaBalpha phosphorylation, ubiquitination, and degradation, which finally leads to NF-kappaB activation. NF-kappaB activation is followed by the expression of various genes that can suppress the apoptosis induced by TNF-alpha. Through recruitment of TRAF2, TNF-alpha has also been shown to activate various mitogen-activated protein kinases (MAPK) including the c-jun N-terminal kinases (JNK) p38 MAPK and p42/p44 MAPK. TRAF2 is also essential for the TNF-alpha-induced activation of AKT, another cell-survival signaling pathway (Figure 1). Thus TNFRI activates both apoptosis and cell survival signaling pathways simultaneously (9-11).

In contrast to TNFR1, the cytoplasmic domain of TNFR2 lacks the death domain and binds TRAF1 and TRAF2 directly. Through activation of JNK, TNF-alpha activates AP-1, another redox-sensitive transcription factor. Gene-deletion studies have shown that TNFR2 can also activate NF-kappaB, JNK, p38 MAPK and p42/p44 MAPK (12). TNFR2 can also mediate TNF-alpha-induced apoptosis (13). Because TNFR2 cannot recruit TRADD-FADD-FLICE, how TNFR2 mediates apoptosis is not understood. Various pieces of evidence suggest that homotrimeric TNF-alpha binds to homotrimeric TNF-alpha

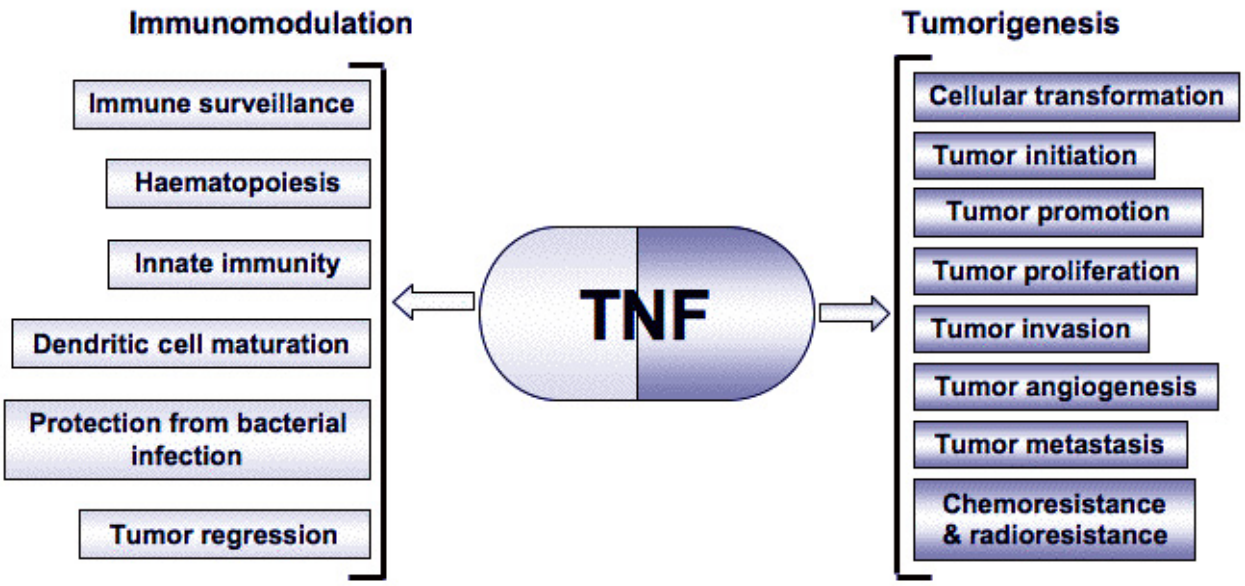

Figure 2. Dual role of TNF in immunomodulation and tumorigenesis.

receptor to mediate its signals (14). TNF-alpha receptor deletion studies have provided evidence that this receptor communicates with receptors for other ligands, including receptor activator of NF-kappaB ligand (RANKL, a member of the TNF superfamily), and endotoxin (15, 16).

4. ROLE OF TNF-ALPHA IN INFLAMMATION

Inflammation is the physiologic response to injury caused by wounding, chemical irritation/damage, or infection. Acute inflammation initiates a cascade of cytokines and chemokines that attract immune and non-immune cells, mainly neutrophils, to infiltrate disrupted and damaged tissue. The process of acute inflammation is usually self-limiting because the production of pro-inflammatory cytokines is replaced by anti-inflammatory cytokines as healing progresses. However, in chronic inflammation other mononuclear cells, macrophages, lymphocytes, and plasma cells are found in addition to neutrophils, and active tissue destruction and repair proceed simultaneously (17-19). Acute inflammation is therapeutic in nature and counteracts cancer (20).

However, when inflammation becomes chronic or lasts too long, it can prove harmful and may lead to disease. How is inflammation diagnosed and its biomarkers is not fully understood, however, the role of pro-inflammatory cytokines, chemokines, adhesion molecules and inflammatory enzymes have been linked with chronic inflammation (21). Various clinical and epidemiologic studies have suggested a strong association between chronic inflammation, and cancer (21, 22). For example, there are strong associations between alcohol abuse, which leads to inflammation of the liver and pancreas, and cancers of these organs. Cigarette smoking, asbestos exposure, and silica exposure are each associated with inflammation of the lung and lung carcinoma; inflammatory bowel disease (IBD) is associated with colon cancer; infection with *Helicobacter pylori* is associated with gastric carcinoma; chronic viral hepatitis is associated with liver cancer; infection with *Schistosoma* spp. is associated with bladder and colon carcinoma; infection with some strains of HPV is associated with cervical cancer; and infection with EBV is associated with Burkitt lymphoma and nasopharyngeal carcinoma (18-21, 23). Chronic inflammation has been linked to various steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis (Figure 2). Recent data from mouse models of human cancer have established that inflammation, which orchestrates the tumor microenvironment, is a critical component of both tumor promotion and tumor progression (24-26).

The pro-inflammatory effects of TNF-alpha are primarily due to its ability to activate NF-kappaB. Almost all cell types, when exposed to TNF-alpha, activate NF-kappaB, leading to the expression of inflammatory genes. Over 400 genes have been identified that are regulated by NF-kappaB activation. These include cyclooxygenase-2 (COX-2), lipoxygenase-2 (LOX-2), cell-adhesion molecules, antiapoptotic proteins, inflammatory cytokines, chemokines, and inducible nitric oxide synthase (iNOS). TNF-alpha produced by tumor cells or inflammatory cells in the tumor microenvironment can promote tumor cell survival through the induction of genes encoding NF-kappaB-dependent antiapoptotic molecules (27-29). The present review will discuss the role played by TNF-alpha in steps leading to formation of tumors and their metastasis.

5. ROLE OF TNF-ALPHA IN TUMORIGENESIS

TNF-alpha is a critical component of effective immune surveillance and is required for proper proliferation and function of NK cells, T cells, B cells, macrophages, and

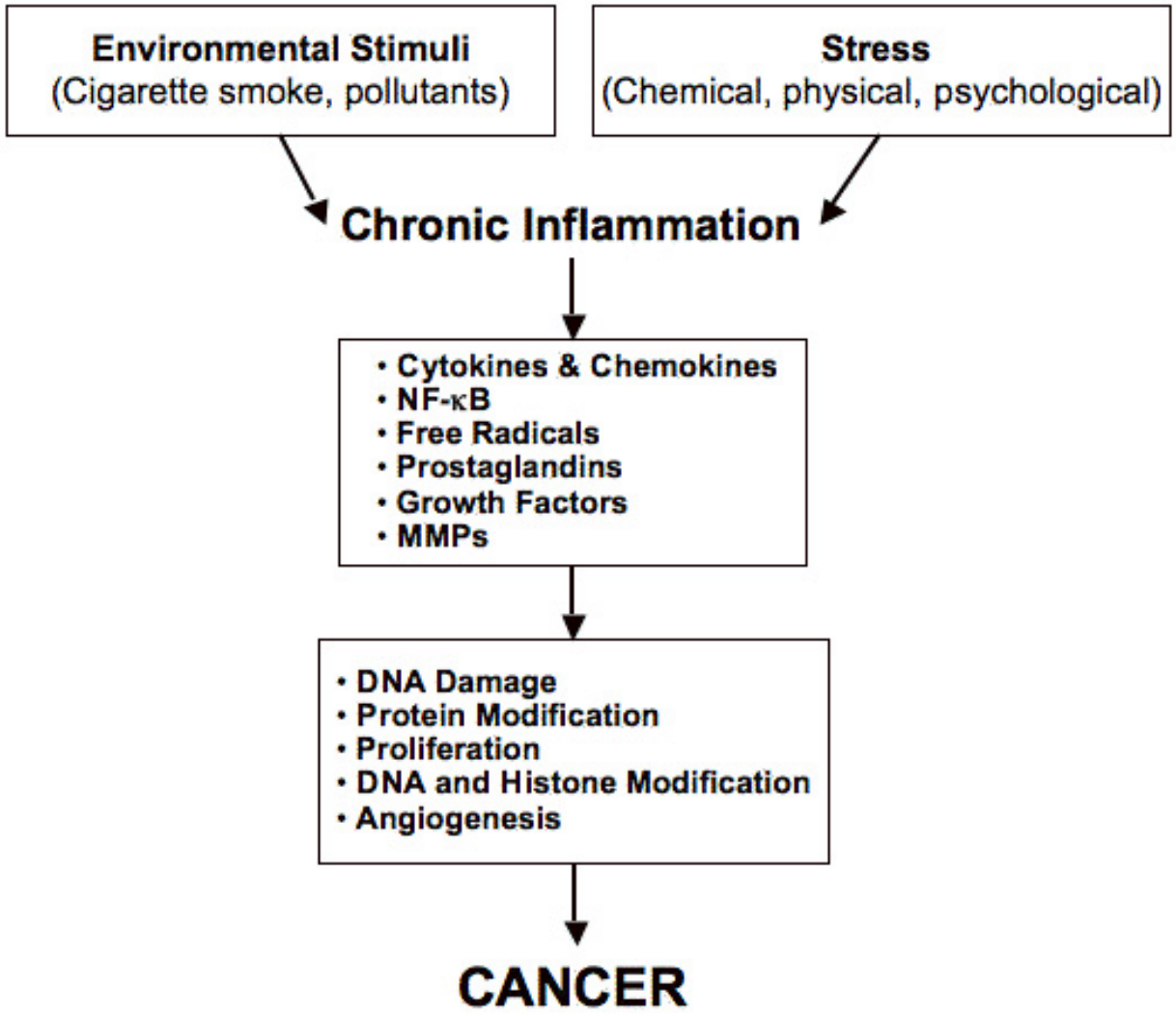

Figure 3. A scheme for inflammation induces cancer.

dendritic cells. However, when dysregulated TNF-alpha has been linked to a wide variety of cancers (30-32). The role of TNF-alpha has been linked to all steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis, as shown in Figure 3 and outlined below.

5.1. TNF-alpha can induce tumor initiation and promotion

A number of reports indicate that TNF-alpha induces tumor initiation and tumor promotion (5, 21, 32). Komori's group reported that human TNF-alpha is 1000 times more effective than the chemical tumor promoters okadaic acid and 12-O-tetradecanoylphorbol-13-acetate in inducing cancer. Once initiated with these chemical carcinogens and exposed for 2 weeks to TNF-alpha, BALB/3T3 cells underwent transformation and yielded tumors in nude mice (33). The essential role of TNF-alpha in tumor promotion has also been demonstrated using TNF-alpha-deficient mice. Specifically, okadaic acid did not show any tumor-promoting activity in TNF-alpha $^{-/-}$ mice after up to 19 weeks of tumor promotion, whereas okadaic acid induced strong tumor-promoting activity in TNF-alpha $^{+/+}$ mice. Tumor development in TPA-treated TNF-alpha $^{-/-}$ mice was delayed, and both the average number of tumors per mouse and the tumor size were dramatically reduced compared with results for TNF-alpha $^{+/+}$ CD-1 mice (34). Similarly, in a model of chemically induced liver cancer, TNF-alpha production by hepatocytes was implicated in tumor development (35). All these reports establish that TNF-alpha plays a critical role in tumor promotion.

5.2. Tumor cells produce TNF-alpha and mediate proliferation

Although initially thought to be a product of macrophages, TNF-alpha has now been shown to be produced by a wide variety of tumor cells, including those of B cell lymphoma (36, 37), cutaneous T cell lymphoma (38), megakaryoblastic leukemia (39), adult T cell leukemia (40), AML, CLL (41), ALL (42), breast carcinoma (43), colon carcinoma, lung carcinoma,

squamous cell carcinoma, pancreatic cancer (44, 45), ovarian carcinoma (46-48), cervical epithelium (49), glioblastoma (50), and neuroblastoma (38, 41, 42, 46, 50, 51). In most of these cells, TNF-alpha acts as an autocrine growth factor (36-51); however, in some cell types TNF-alpha induces the expression of other growth factors, which mediate proliferation of tumors. For instance, in cervical cells TNF-alpha induces amphiregulin, which induces the proliferation of cells (49), whereas in pancreatic cells TNF-alpha induces the expression of epidermal growth factor receptor (EGFR) and transforming growth factor (TGF-alpha), both of which mediate proliferation (45).

TNF-alpha is expressed by human ovarian carcinoma *in vivo* (47). Four of five ascites fluid specimens and tissue sections of 16 of 20 patients were shown to be positive for TNF-alpha. The gene for TNF-alpha was expressed in 45 of 63 biopsies of human epithelial ovarian cancer (48). In serous tumors, there was a positive correlation between the level of TNF-α expression and tumor grade. TNF-alpha mRNA was found in epithelial tumor cells and infiltrating macrophages, whereas TNF-alpha protein localized primarily to a subpopulation of macrophages within and in close proximity to tumor areas. mRNA and protein for the p55 TNF-alpha receptor gene localized to the tumor epithelium and tumor but not to stromal macrophages. Cells expressing TNF mRNA were also found in ovarian cancer ascites, and TNF-alpha protein was detected in some ascetic fluids. In 2 of 12 biopsies of normal ovary, TNF-alpha mRNA was detected in a minority of cells in the thecal layer of the corpus luteum. The coexpression of TNF-alpha and its receptor in ovarian cancer biopsies suggests the capacity for autocrine/paracrine action. TNF-alpha antagonists may have therapeutic potential in this malignancy.

TNF-alpha is constitutively produced by B-cell chronic lymphocytic leukemia (B-CLL) and hairy cell leukemia (HCL) cells and may play a relevant role in these diseases (52). These conclusions are based on the presence of circulating levels of TNF-alpha in the serum of 20 of the 24 patients tested, with undetectable values found in normal sera. When primary B-CLL cells were incubated in the presence of an anti-TNF-alpha antibody, increased thymidine uptake was documented. Thus, TNF-alpha plays a regulatory role in the progression of the neoplastic clone in B-cell chronic lymphoproliferative disorders.

TNF-alpha serves predominantly as a mitogen for Mo7e cell proliferation and does not induce Mo7e cell differentiation (39). Coincubation with both TNF-alpha and anti-TNF-alpha neutralizing antibody completely abolishes the TNF-alpha-induced proliferation of Mo7e cells. We found that TNF-alpha is an autocrine growth factor for human glioblastoma tumor cells and that suppression of TNF-alpha secretion will inhibit the growth of this tumor (50). TNF-alpha also acts as an autocrine growth factor for neuroblastoma (51). There is also an evidence of a crosstalk between inflammatory cytokines and growth factor pathways. TNF-alpha decreased the expression of ERBB2 mRNA by stimulating p55 TNF-alpha receptors of pancreatic tumor cells (44). This decrease contrasts with an increase in epidermal growth factor receptor (EGFR) mRNA. This decrease of ERBB2 is a singular example of a modulation of this growth factor receptor by TNF-alpha. Overexpression of ERBB2 has been reported to cause resistance to TNF and other cytotoxic cytokines. TNF-mediated downregulation of ERBB2 in pancreatic tumor cells is accompanied by an increase in growth inhibition at low doses of TNF-alpha. TNF-alpha has been shown to induce the expression of TGF-alpha and EGFR in human pancreatic cancer cells. The simultaneous induction of a ligand/receptor system by TNF-alpha suggests that this cytokine modulates autocrine growth-regulatory pathways in pancreatic cancer cells (45).

The mechanism of TNF-alpha production by tumor cells is not fully understood. TNF-alpha polymorphism is associated with increased susceptibility to development of ATL/lymphoma in human T-lymphotropic virus type 1 (HTLV-1) carriers (40). It has been found that the frequency of the TNF-alpha-857T allele, reported to be associated with high transcriptional activity of the promoter/enhancer region of the TNF-alpha gene, was enriched in individuals with ATL compared with healthy carriers. Genetic polymorphism leading to increased TNF-alpha production may enhance susceptibility to ATL among HTLV-1 carriers.

5.3. TNF can induce invasion and angiogenesis of tumor cells

Although loss of cell-cell adhesion and gain of invasive properties play a crucial role in malignant progression of epithelial tumors, the molecular signals that trigger these processes have not been fully elucidated. TNF-alpha has been shown to confer an invasive, transformed phenotype on mammary epithelial cells (43). This suggests that pro-inflammatory cytokines disrupt epithelial-cell adhesion and promote cell migration. This suggests an essential role for MMPs and alpha2beta1 integrins in the invasive response of 31EG4-2A4 cells to TNF-alpha. TNF-alpha induces angiogenic factor upregulation in malignant glioma cells (53). This upregulation in turn promotes angiogenesis and tumor progression. There is a marked upregulation (RNA and protein) of TNF-alpha, IL-8, and, to a lesser extent, vascular endothelial growth factor (VEGF) in U251 glioma cells after stimulation with TNF-alpha.

Cellular motility is a critical function in embryonic development, tissue repair, and tumor invasion. TNF-alpha stimulates epithelial tumor cell motility (54). TNF-alpha could enhance invasiveness of some carcinomas or stimulate epithelial wound healing *in vivo*. Whether TNF-alpha-induced angiogenesis is mediated through TNF-alpha itself or indirectly through other TNF-alpha induced angiogenesis-promoting factors has been investigated (55). The involvement of IL-8, VEGF, and betaFGF has been documented in TNF-alpha-dependent angiogenesis. TNF-alpha-dependent

tubular morphogenesis in vascular endothelial cells was inhibited by the administration of anti-IL-8, anti-VEGF, and anti-betaFGF antibodies, and co-administration of all three antibodies almost completely abrogated tubular formation. Administration of an anti-IL-8 or anti-VEGF antibody also blocked TNF-alpha-induced neovascularization in the rabbit cornea *in vivo*. Thus, angiogenesis by TNF-alpha appears to be modulated through various angiogenic factors, both *in vitro* and *in vivo*, and this pathway is controlled through paracrine and/or autocrine mechanisms.

Macrophages are important in the induction of new blood vessel growth during wound repair, inflammation, and tumor growth. It has been demonstrated that macrophage-induced angiogenesis is mediated by TNF-alpha (56). *In vivo*, TNF-alpha induces capillary blood vessel formation in the rat cornea and the developing chick chorioallantoic membrane at very low doses. *In vitro*, TNF-alpha stimulates chemotaxis of bovine adrenal capillary endothelial cells and induces cultures of these cells grown on type-1 collagen gels to form capillary tube-like structures. The angiogenic activity produced by activated murine peritoneal macrophages is completely neutralized by a polyclonal antibody to TNF-alpha, suggesting that immunological features are common to TNF-alpha and the protein responsible for macrophage-derived angiogenic activity. In inflammation and wound repair, TNF-alpha could augment repair by stimulating new blood vessel growth; TNF-alpha might stimulate tumor development by promoting vessel growth in the tumor.

5.4. Role of TNF-alpha and its receptor in cancer development

The role of both TNF-alpha and its receptors has been examined in cancer development. Various approaches, including genetic deletion, transgenic models, antibodies and soluble receptors as decoys, have been used to gain insight into the role of TNF-alpha in tumor development. Studies have shown that tumor necrotic factor receptor (TNFR-1)-mediated signaling is required for skin cancer development induced by NF-kappaB inhibition (57). This suggests a critical role of local TNFR1-mediated signaling and associated inflammatory response cooperating with repressed keratinocyte NF-kappaB signaling in driving skin cancer development. An essential role of TNFR p55 has been determined in the liver metastasis of intrasplenic administration of colon 26 cells (58). TNFR p55-mediated signals can upregulate both VCAM-1 expression in the liver and subsequent liver metastasis after intrasplenic tumor injection. Two-stage carcinogenesis experiments on TNF$^{-/-}$ mice have shown that TNF-alpha is the key cytokine for tumor promotion in mouse skin and, very possibly, for carcinogenesis in humans as well (34).

Pretreatment with the four cancer-preventive agents -- sarcophytol A, canventol, (-)-epigallo-catechin gallate, and tamoxifen -- inhibited TNF-alpha mRNA expression and TNF-alpha release in BALB/3T3 cells induced by the tumor promoter okadaic acid while enhancing the expression of early response genes (c-jun, junB, c-fos, and fosB), thus suggesting that inhibition of TNF-alpha mRNA expression and its release is a new process of cancer prevention (59). It has been further shown that a pro-inflammatory cytokine is required for de novo carcinogenesis and that TNF-alpha is important to the early stages of tumor promotion (60). TNF-alpha$^{-/-}$ mice were resistant to the development of benign and malignant skin tumors, whether induced by initiation with DMBA and promotion with TPA or by repeated dosing with DMBA. TNF-alpha$^{-/-}$ mice developed 5-10% the number of tumors developed by wild-type mice during initiation/promotion and 25% of those in wild-type mice after repeated carcinogen treatment. TNF-alpha could influence tumor and stromal cells during tumor development. The early stages of TPA promotion are characterized by keratinocyte hyperproliferation and inflammation. These were diminished in TNF-alpha$^{-/-}$ mice. Deletion of a TNF-alpha-inducible chemokine also conferred some resistance to skin tumor development. TNF-alpha had little influence on later stages of carcinogenesis, as tumors in wild-type and TNF-alpha$^{-/-}$ mice had similar rates of malignant progression. Strategies that neutralize TNF-alpha production may be useful in cancer treatment and prevention.

Hepatic stem cells (oval cells) proliferate within the liver after exposure to a variety of hepatic carcinogens and can generate both hepatocytes and bile duct cells. Oval cell proliferation is commonly seen in the preneoplastic stages of liver carcinogenesis, often accompanied by an inflammatory response. TNF-alpha is also important in liver regeneration and hepatocellular growth. It has been demonstrated that TNF-alpha is upregulated during oval cell proliferation induced by a choline-deficient, ethionine-supplemented diet and that it is expressed by oval cells (35). In TNFR1-knockout mice, oval cell proliferation is substantially impaired and tumorigenesis is reduced. Oval cell proliferation is unchanged in TNFR2-knockout mice. These findings demonstrate that TNF-alpha signaling participates in the proliferation of oval cells during the preneoplastic phase of liver carcinogenesis and that loss of signaling through the TNFR1 reduces the incidence of tumor formation. Thus the TNF-alpha inflammatory pathway may be a target for therapeutic intervention during the early stages of liver carcinogenesis.

Endogenous and exogenous TNF-alpha showed enhancement of metastasis in an experimental fibrosarcoma metastasis model (61). A single intraperitoneal injection of recombinant human (rh) TNF-alpha or recombinant mouse (rm) TNF-alpha into mice 5 h before intravenous inoculation of methylcholanthrene-induced fibrosarcoma cells (CFS1) significantly enhanced the number of metastases in the lung. Neutralization of endogenous tumor-induced TNF-alpha led to a significant decrease of the number of pulmonary metastases. The expression of TNF-alpha by different tumor cell lines results either in tumor suppression or augmented metastasis (62). TNF-alpha

expression can also induce resistance to TNF-alpha in human breast tumor cell lines (63).

6. INHIBITORS OF TNF-ALPHA

On the basis of the above descriptions, TNF blockers have tremendous potential for the treatment of various types of cancers. Several classes of TNF-alpha inhibitors are available, and these are discussed below.

6.1. TNF-alpha antibodies

The best studied of the monoclonal TNF-alpha antibodies is infliximab (Remicade), originally referred to as cA2. Infliximab binds with high specificity and affinity to free and membrane-bound TNF-alpha, which is expressed at the cell surface by activated T cells and macrophages (64). Adalimumab (Humira) is a human monoclonal IgG_1 antibody containing only human peptide sequences. It binds with high specificity and affinity to soluble and membrane-bound TNF-alpha and blocks its interaction with the p55 and p75 cell surface TNF receptors, thereby neutralizing the biological activities of this cytokine (65). However, these antibodies have demonstrated several potentially serious adverse effects that include greater predisposition towards infection, congestive heart failure, neurologic changes (e.g., demyelination), lymphomas, re-exacerbation of latent tuberculosis and problems related to autoimmunity, for example lupus-like syndrome (66).

6.2. Soluble TNF-alpha receptors

In the second approach to TNF-alpha inhibition, soluble TNF receptors have been engineered as fusion proteins in which the extracellular ligand-binding portion of TNFRI or TNFR2 is coupled to a human immunoglobulin-like molecule. Etanercept (Enbrel) is a recombinant human fusion protein that consists of two soluble p75 TNF receptors and the F_c portion of human IgG_1 (67). Etanercept possesses a dimeric structure with high affinity to TNF-alpha, and the linkage to the F_c portion of human IgG produces a longer half-life. Etanercept is better at neutralizing TNF-alpha than is the monomeric soluble p75 receptor. The various side effects observed include lymphomas, re-exacerbation of latent tuberculosis, and problems related to autoimmunity (66). Recent studies indicate that administration of TNF-alpha inhibitors can even lead to psoriasis (68) and contribute to the severity of the disease in paracoccidioidomycosis (69).

Besides p75, TNF-alpha has been shown to bind to p55 receptor with an affinity either equal or even greater than p75 (70). Although soluble p75 receptors clearly can sequester TNF-alpha, very little is known about the ability of the soluble form of the p55 receptor to sequester TNF-alpha *in vivo*.

6.3. Inhibitors of TNF-alpha expression

Several compounds that can inhibit both TNF-alpha expression and synthesis are also available. These include thalidomide ([+]-alpha-phthalimidoglutarimide), which is currently being used for treatment of multiple myeloma (71, 72), and pentoxifylline, used to treat leg pain caused by poor blood circulation (73). Thus these agents may be useful for the treatment of various cancers and autoimmune diseases mediated by TNF-alpha.

6.4. Inhibitors of TNF-alpha oligomerization

Some inhibitors that can suppress oligomerization of TNF-alpha are also known. Steed and coworkers (74) designed a novel dominant-negative variant TNF-alpha protein that rapidly forms heterotrimers with native TNF-alpha to give complexes that neither bind to nor stimulate signaling through TNF-α receptors and thus inactivate TNF-alpha by sequestration. He *et al*. (75) identified another small-molecule inhibitor that promotes subunit disassembly of trimeric TNF-alpha. This compound inhibited TNF-alpha activity in biochemical and cell-based assays, with median inhibitory concentrations of 22 and 4.6 micromolar, respectively. Formation of an intermediate complex between the compound and the intact trimer resulted in a 600-fold accelerated subunit dissociation rate that led to trimer dissociation.

6.5. Inhibitors of TNF-alpha-induced signaling pathways

TNF-alpha activates cell survival signaling pathways, i.e., NF-kappaB, Akt, and MAPK pathways, as well as apoptotic pathways such as JNK, p38, and AP-1. Hence, inhibitors that target these pathways also have potential against various proinflammatory conditions mediated by TNF-alpha. For example, TNF-alpha activates NF-kappaB, which in turn regulates TNF-alpha production (32). Hence various NF-kappaB blockers (both synthetic and natural) are currently available on the market and effective against a wide variety of inflammatory conditions.

7. NATURAL PRODUCTS AS INHIBITORS OF TNF-ALPHA

Numerous plant-derived products have been identified that can suppress TNF-alpha expression from macrophages activated by numerous inflammatory stimuli (76-114, see Table 1). These include curcumin, resveratrol, emodin, silymarin, and others. Thus these products are likely to be useful for the treatment of cancer and autoimmune diseases mediated by TNF-alpha.

8. CONCLUSION

TNF-alpha clearly plays a major role in establishing a link between inflammation and cancer. Because TNF-alpha is also needed for the proper functioning of the immune system, complete suppression of TNF-alpha over a long period is likely to prove harmful. Any chronic inflammatory condition, linked to majority of the inflammatory diseases, could be a potential target for anti-TNF-alpha therapy. Thus the development of inhibitors that are orally active, safe, and inexpensive would have major potential. Because of long-term safety and cost, nutraceuticals derived from fruits and vegetables, that can suppress TNF-alpha expression and TNF-alpha signaling, should be explored clinically for efficacy.

Table 1. A list of natural products that inhibit tumor necrosis factor expression

Name of natural products and their inhibitory mechanism	Ref
ACA and AEA inhibits LPS, cytokine, and amyloid Abeta peptide-induced TNF-alpha expression in THP-1 cell line and antigen-IgE antibody induced TNFα in RBL-2H3 cells in mice	76, 77
Allium sativum inhibits LPS-stimulated TNF-alpha expression in human placental explants	78
Aloe vera inhibits burn induced TNF-alpha expression in rats	79
Aloe barbadensis inhibits UVB irradiation-induced TNF-alpha expression in KB cells	80
Asparagus cochinchinensis inhibits LPS induced TNF-alpha expression in primary cultures of mouse astrocytes	81
Bisdemethoxycurcumin inhibits antigen-IgE induced TNF-alpha expression in RBL-2H3 cells	82
Butein inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	83
Cardamomin inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	84
Curcumin inhibits LPS induced TNF-alpha expression in Mono Mac 6 cells and in MCL cells	85, 86
Diphenyl dimethyl bicarboxylate inhibits concanavalin A induced TNF-alpha expression in mice	87
Emodin inhibits IL-1beta and IL-6 induced TNF-alpha expression in human mesangial cells	88
Epigallocatechin gallate inhibits bacterial infection induced TNF-alpha expression in MH-S cells	89
F022 inhibits LPS induced TNF-alpha in murine peritoneal macrophages	90
Ginkgolide B inhibits LPS induced TNF-alpha production in mouse peritoneal macrophages and in RAW 264.7 cells	91, 92
2'-hydroxychalcone inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	93, 94
Hypoestoxide inhibits LPS induced TNF-alpha expression in normal human peripheral blood mononuclear cells	95
Inula britannica inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	96
Lonicera japonica inhibits trypsin induced TNF-alpha expression HMC-1	97
Neolignans and lignans inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	98
Patridoids I, II and IIA inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	99
Phthalide lactone inhibits LPS induced TNF-alpha expression in monocytes	100
Phloroglucinol derivatives inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	101
Platycodin D and D3 inhibits LPS and rIFN-gamma induced TNF-alpha expression in RAW 264.7 cells	102
Phlebodium decumanum inhibits LPS and IFN-gamma induced TNF-alpha expression in peripheral blood mononuclear cells	103
Phyllanthus amarus inhibits LPS induced TNF-alpha expression in RAW 264.7 cells [104]	
Polygala tenuifolia inhibits LPS induced TNF-alpha expression in primary cultures of mouse astrocytes	105
Resveratrol inhibits LPS induced TNF-alpha expression in microglia	106
14,15-secopregnane derivatives, argelosides K-O (1-5), inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	107
Tanacetum microphyllum in LPS induced TNF-alpha expression mouse peritoneal macrophages	108
Taraxacum officinale inhibits LPS induced TNF-alpha expression in rat astrocytes	109
Delta(9)-tetrahydrocanabinoid acid inhibits LPS induced TNF-alpha expression in U937 macrophages and peripheral blood macrophages	110
Theobroma cacao inhibits LPS and IFN-gamma induced TNFα expression in RAW 264.7 and NR8383 cells	111
Uncaria guianensis inhibits LPS induced TNF-alpha expression in RAW 264.7 cells	112
Yakuchinone A and B inhibits 12-O-tetradecanoylphorbol-13-acetate induced TNF-alpha expression in mouse skin	113
Zingiber officinale inhibits LPS, cytokine, and amyloid Abeta peptide-induced TNF-alpha expression in THP-1 cells	79
Zostera japonica inhibits LPS induced TNF-alpha expression in J774A.1 murine macrophages	114

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10. REFERENCES

1. B. B. Aggarwal, B. Moffat and R. N. Harkins: Human lymphotoxin. Production by a lymphoblastoid cell line, purification, and initial characterization. *J Biol Chem*, 259(1), 686-91 (1984)

2. R. Kurzrock, M. G. Rosenblum, S. A. Sherwin, A. Rios, M. Talpaz, J. R. Quesada and J. U. Gutterman: Pharmacokinetics, single-dose tolerance, and biological activity of recombinant gamma-interferon in cancer patients. *Cancer Res*, 45(6), 2866-72 (1985)

3. B. Beutler, D. Greenwald, J. D. Hulmes, M. Chang, Y. C. Pan, J. Mathison, R. Ulevitch and A. Cerami: Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature*, 316(6028), 552-4 (1985)

4. B. B. Aggarwal: Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol*, 3(9), 745-56 (2003)

5. B. B. Aggarwal, S. Shishodia, K. Ashikawa and A. C. Bharti: The role of TNF and its family members in

inflammation and cancer: lessons from gene deletion. *Curr Drug Targets Inflamm Allergy*, 1(4), 327-41 (2002)

6. L. A. Tartaglia, D. Pennica and D. V. Goeddel: Ligand passing: the 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. *J Biol Chem*, 268(25), 18542-8 (1993)

7. M. Grell, E. Douni, H. Wajant, M. Lohden, M. Clauss, B. Maxeiner, S. Georgopoulos, W. Lesslauer, G. Kollias, K. Pfizenmaier and P. Scheurich: The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell*, 83(5), 793-802 (1995)

8. S. Nagata and P. Golstein: The Fas death factor. *Science*, 267(5203), 1449-56 (1995)

9. B. G. Darnay and B. B. Aggarwal: Early events in TNF signaling: a story of associations and dissociations. *J Leukoc Biol*, 61(5), 559-66 (1997)

10. A. Bhardwaj and B. B. Aggarwal: Receptor-mediated choreography of life and death. *J Clin Immunol*, 23(5), 317-32 (2003)

11. B. B. Aggarwal and Y. Takada: Pro-apototic and anti-apoptotic effects of tumor necrosis factor in tumor cells. Role of nuclear transcription factor NF-kappaB. *Cancer Treat Res*, 126, 103-27 (2005)

12. A. Mukhopadhyay, J. Suttles, R. D. Stout and B. B. Aggarwal: Genetic deletion of the tumor necrosis factor receptor p60 or p80 abrogates ligand-mediated activation of nuclear factor-kappa B and of mitogen-activated protein kinases in macrophages. *J Biol Chem*, 276(34), 31906-12 (2001)

13. V. Haridas, B. G. Darnay, K. Natarajan, R. Heller and B. B. Aggarwal: Overexpression of the p80 TNF receptor leads to TNF-dependent apoptosis, nuclear factor-kappa B activation, and c-Jun kinase activation. *J Immunol*, 160(7), 3152-62 (1998)

14. P. Ameloot, W. Declercq, W. Fiers, P. Vandenabeele and P. Brouckaert: Heterotrimers formed by tumor necrosis factors of different species or muteins. *J Biol Chem*, 276(29), 27098-103 (2001)

15. Y. Takada and B. B. Aggarwal: Genetic deletion of the tumor necrosis factor receptor p60 or p80 sensitizes macrophages to lipopolysaccharide-induced nuclear factor-kappa B, mitogen-activated protein kinases, and apoptosis. *J Biol Chem*, 278(26), 23390-7 (2003)

16. Y. Takada and B. B. Aggarwal: Evidence that genetic deletion of the TNF receptor p60 or p80 in macrophages modulates RANKL-induced signaling. *Blood*, 104(13), 4113-21 (2004)

17. D. Hanahan and R. A. Weinberg: The hallmarks of cancer. *Cell*, 100(1), 57-70 (2000)

18. L. M. Coussens and Z. Werb: Inflammation and cancer. *Nature*, 420(6917), 860-7 (2002)

19. E. Shacter and S. A. Weitzman: Chronic inflammation and cancer. *Oncology (Williston Park)*, 16(2), 217-26, 229; discussion 230-2 (2002)

20. S. P. Hussain, L. J. Hofseth and C. C. Harris: Radical causes of cancer. *Nat Rev Cancer*, 3(4), 276-85 (2003)

21. B. B. Aggarwal, S. Shishodia, S. K. Sandur, M. K. Pandey and G. Sethi: Inflammation and cancer: how hot is the link? *Biochem Pharmacol*, 72(11), 1605-21 (2006)

23. H. Lu, W. Ouyang and C. Huang: Inflammation, a key event in cancer development. *Mol Cancer Res*, 4(4), 221-33 (2006)

23. J. G. Fox and T. C. Wang: Inflammation, atrophy, and gastric cancer. *J Clin Invest*, 117(1), 60-9 (2007)

24. F. Balkwill, K. A. Charles and A. Mantovani: Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell*, 7(3), 211-7 (2005)

25. M. Karin: Nuclear factor-kappaB in cancer development and progression. *Nature*, 441(7092), 431-6 (2006)

26. M. Karin and F. R. Greten: NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol*, 5(10), 749-59 (2005)

27. B. B. Aggarwal: Nuclear factor-kappaB: the enemy within. *Cancer Cell*, 6(3), 203-8 (2004)

28. S. Shishodia and B. B. Aggarwal: Nuclear factor-kappaB activation mediates cellular transformation, proliferation, invasion angiogenesis and metastasis of cancer. *Cancer Treat Res*, 119, 139-73 (2004)

29. K. S. Ahn and B. B. Aggarwal: Transcription factor NF-kappaB: a sensor for smoke and stress signals. *Ann N Y Acad Sci*, 1056, 218-33 (2005)

30. S. Perwez Hussain and C. C. Harris: Inflammation and cancer: An ancient link with novel potentials. *Int J Cancer*, 121(11), 2373-80 (2007)

31. W. W. Lin and M. Karin: A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest*, 117(5), 1175-83 (2007)

32. B. B. Aggarwal, S. Shishodia, Y. Takada, D. Jackson-Bernitsas, K. S. Ahn, G. Sethi and H. Ichikawa: TNF blockade: an inflammatory issue. *Ernst Schering Res Found Workshop*(56), 161-86 (2006)

33. A. Komori, J. Yatsunami, M. Suganuma, S. Okabe, S. Abe, A. Sakai, K. Sasaki and H. Fujiki: Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformation. *Cancer Res*, 53(9), 1982-5 (1993)

34. M. Suganuma, S. Okabe, M. W. Marino, A. Sakai, E. Sueoka and H. Fujiki: Essential role of tumor necrosis factor alpha (TNF-alpha) in tumor promotion as revealed by TNF-alpha-deficient mice. *Cancer Res*, 59(18), 4516-8 (1999)

35. B. Knight, G. C. Yeoh, K. L. Husk, T. Ly, L. J. Abraham, C. Yu, J. A. Rhim and N. Fausto: Impaired preneoplastic changes and liver tumor formation in tumor necrosis factor receptor type 1 knockout mice. *J Exp Med*, 192(12), 1809-18 (2000)

36. W. Digel, M. Stefanic, W. Schoniger, C. Buck, A. Raghavachar, N. Frickhofen, H. Heimpel and F. Porzsolt: Tumor necrosis factor induces proliferation of neoplastic B cells from chronic lymphocytic leukemia. *Blood*, 73(5), 1242-6 (1989)

37. W. Digel, W. Schoniger, M. Stefanic, H. Janssen, C. Buck, M. Schmid, A. Raghavachar and F. Porzsolt: Receptors for tumor necrosis factor on neoplastic B cells from chronic lymphocytic leukemia are expressed *in vitro* but not *in vivo*. *Blood*, 76(8), 1607-13 (1990)

38. D. K. Giri and B. B. Aggarwal: Constitutive activation of NF-kappaB causes resistance to apoptosis in human cutaneous T cell lymphoma HuT-78 cells. Autocrine role of tumor necrosis factor and reactive oxygen intermediates. *J Biol Chem*, 273(22), 14008-14 (1998)

39. R. Y. Liu, C. Fan, S. Mitchell, Q. Chen, J. Wu and K. S. Zuckerman: The role of type I and type II tumor necrosis factor (TNF) receptors in the ability of TNF-alpha to transduce a proliferative signal in the human megakaryoblastic leukemic cell line Mo7e. *Cancer Res*, 58(10), 2217-23 (1998)

40. K. Tsukasaki, C. W. Miller, T. Kubota, S. Takeuchi, T. Fujimoto, S. Ikeda, M. Tomonaga and H. P. Koeffler: Tumor necrosis factor alpha polymorphism associated with increased susceptibility to development of adult T-cell leukemia/lymphoma in human T-lymphotropic virus type 1 carriers. *Cancer Res*, 61(9), 3770-4 (2001)

41. A. S. Duncombe, H. E. Heslop, M. Turner, A. Meager, R. Priest, T. Exley and M. K. Brenner: Tumor necrosis factor mediates autocrine growth inhibition in a chronic leukemia. *J Immunol*, 143(11), 3828-34 (1989)

42. O. Elbaz and L. A. Mahmoud: Tumor necrosis factor and human acute leukemia. *Leuk Lymphoma*, 12(3-4), 191-5 (1994)

43. R. Montesano, P. Soulie, J. A. Eble and F. Carrozzino: Tumour necrosis factor alpha confers an invasive, transformed phenotype on mammary epithelial cells. *J Cell Sci*, 118(Pt 15), 3487-500 (2005)

44. H. Kalthoff, C. Roeder, J. Gieseking, I. Humburg and W. Schmiegel: Inverse regulation of human ERBB2 and epidermal growth factor receptors by tumor necrosis factor alpha. *Proc Natl Acad Sci U S A*, 90(19), 8972-6 (1993)

45. W. Schmiegel, C. Roeder, J. Schmielau, U. Rodeck and H. Kalthoff: Tumor necrosis factor alpha induces the expression of transforming growth factor alpha and the epidermal growth factor receptor in human pancreatic cancer cells. *Proc Natl Acad Sci U S A*, 90(3), 863-7 (1993)

46. S. Wu, C. M. Boyer, R. S. Whitaker, A. Berchuck, J. R. Wiener, J. B. Weinberg and R. C. Bast, Jr.: Tumor necrosis factor alpha as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis factor alpha expression. *Cancer Res*, 53(8), 1939-44 (1993)

47. H. Takeyama, N. Wakamiya, C. O'Hara, K. Arthur, J. Niloff, D. Kufe, K. Sakarai and D. Spriggs: Tumor necrosis factor expression by human ovarian carcinoma *in vivo*. *Cancer Res*, 51(16), 4476-80 (1991)

48. M. S. Naylor, G. W. Stamp, W. D. Foulkes, D. Eccles and F. R. Balkwill: Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. *J Clin Invest*, 91(5), 2194-206 (1993)

49. C. D. Woodworth, E. McMullin, M. Iglesias and G. D. Plowman: Interleukin 1 alpha and tumor necrosis factor alpha stimulate autocrine amphiregulin expression and proliferation of human papillomavirus-immortalized and carcinoma-derived cervical epithelial cells. *Proc Natl Acad Sci U S A*, 92(7), 2840-4 (1995)

50. B. B. Aggarwal, L. Schwarz, M. E. Hogan and R. F. Rando: Triple helix-forming oligodeoxyribonucleotides targeted to the human tumor necrosis factor (TNF) gene inhibit TNF production and block the TNF-dependent growth of human glioblastoma tumor cells. *Cancer Res*, 56(22), 5156-64 (1996)

51. E. Goillot, V. Combaret, R. Ladenstein, D. Baubet, J. Y. Blay, T. Philip and M. C. Favrot: Tumor necrosis factor as an autocrine growth factor for neuroblastoma. *Cancer Res*, 52(11), 3194-200 (1992)

52. R. Foa, M. Massaia, S. Cardona, A. G. Tos, A. Bianchi, C. Attisano, A. Guarini, P. F. di Celle and M. T. Fierro: Production of tumor necrosis factor-alpha by B-cell chronic lymphocytic leukemia cells: a possible regulatory role of TNF in the progression of the disease. *Blood*, 76(2), 393-400 (1990)

53. L. B. Nabors, E. Suswam, Y. Huang, X. Yang, M. J. Johnson and P. H. King: Tumor necrosis factor alpha induces angiogenic factor up-regulation in malignant glioma cells: a role for RNA stabilization and HuR. *Cancer Res*, 63(14), 4181-7 (2003)

54. E. M. Rosen, I. D. Goldberg, D. Liu, E. Setter, M. A. Donovan, M. Bhargava, M. Reiss and B. M. Kacinski:

Tumor necrosis factor stimulates epithelial tumor cell motility. *Cancer Res*, 51(19), 5315-21 (1991)

55. S. Yoshida, M. Ono, T. Shono, H. Izumi, T. Ishibashi, H. Suzuki and M. Kuwano: Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. *Mol Cell Biol*, 17(7), 4015-23 (1997)

56. S. J. Leibovich, P. J. Polverini, H. M. Shepard, D. M. Wiseman, V. Shively and N. Nuseir: Macrophage-induced angiogenesis is mediated by tumour necrosis factor-alpha. *Nature*, 329(6140), 630-2 (1987)

57. M. H. Lind, B. Rozell, R. P. Wallin, M. van Hogerlinden, H. G. Ljunggren, R. Toftgard and I. Sur: Tumor necrosis factor receptor 1-mediated signaling is required for skin cancer development induced by NF-kappaB inhibition. *Proc Natl Acad Sci U S A*, 101(14), 4972-7 (2004)

58. H. Kitakata, Y. Nemoto-Sasaki, Y. Takahashi, T. Kondo, M. Mai and N. Mukaida: Essential roles of tumor necrosis factor receptor p55 in liver metastasis of intrasplenic administration of colon 26 cells. *Cancer Res*, 62(22), 6682-7 (2002)

59. M. Suganuma, S. Okabe, E. Sueoka, N. Iida, A. Komori, S. J. Kim and H. Fujiki: A new process of cancer prevention mediated through inhibition of tumor necrosis factor alpha expression. *Cancer Res*, 56(16), 3711-5 (1996)

60. R. J. Moore, D. M. Owens, G. Stamp, C. Arnott, F. Burke, N. East, H. Holdsworth, L. Turner, B. Rollins, M. Pasparakis, G. Kollias and F. Balkwill: Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. *Nat Med*, 5(7), 828-31 (1999)

61. P. Orosz, B. Echtenacher, W. Falk, J. Ruschoff, D. Weber and D. N. Mannel: Enhancement of experimental metastasis by tumor necrosis factor. *J Exp Med*, 177(5), 1391-8 (1993)

62. Z. Qin, S. Kruger-Krasagakes, U. Kunzendorf, H. Hock, T. Diamantstein and T. Blankenstein: Expression of tumor necrosis factor by different tumor cell lines results either in tumor suppression or augmented metastasis. *J Exp Med*, 178(1), 355-60 (1993)

63. D. Spriggs, K. Imamura, C. Rodriguez, J. Horiguchi and D. W. Kufe: Induction of tumor necrosis factor expression and resistance in a human breast tumor cell line. *Proc Natl Acad Sci U S A*, 84(18), 6563-6 (1987)

64. R. Maini, E. W. St Clair, F. Breedveld, D. Furst, J. Kalden, M. Weisman, J. Smolen, P. Emery, G. Harriman, M. Feldmann and P. Lipsky: Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet*, 354(9194), 1932-9 (1999)

65. E. C. Keystone, A. F. Kavanaugh, J. T. Sharp, H. Tannenbaum, Y. Hua, L. S. Teoh, S. A. Fischkoff and E. K. Chartash: Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. *Arthritis Rheum*, 50(5), 1400-11 (2004)

66. U. Hasan: Tumour necrosis factor inhibitors--what we need to know. *N Z Med J*, 119(1246), U2336 (2006)

67. J. M. Bathon, R. W. Martin, R. M. Fleischmann, J. R. Tesser, M. H. Schiff, E. C. Keystone, M. C. Genovese, M. C. Wasko, L. W. Moreland, A. L. Weaver, J. Markenson and B. K. Finck: A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. *N Engl J Med*, 343(22), 1586-93 (2000)

68. R. Ubriani and A. S. Van Voorhees: Onset of psoriasis during treatment with TNF-{alpha} antagonists: a report of 3 cases. *Arch Dermatol*, 143(2), 270-2 (2007)

69. C. L. Corvino, R. L. Mamoni, G. Z. Fagundes and M. H. Blotta: Serum interleukin-18 and soluble tumour necrosis factor receptor 2 are associated with disease severity in patients with paracoccidioidomycosis. *Clin Exp Immunol*, 147(3), 483-90 (2007)

70. M. Higuchi and B. B. Aggarwal: Modulation of two forms of tumor necrosis factor receptors and their cellular response by soluble receptors and their monoclonal antibodies. *J Biol Chem*, 267(29), 20892-9 (1992)

71. S. Majumdar, B. Lamothe and B. B. Aggarwal: Thalidomide suppresses NF-kappa B activation induced by TNF and H2O2, but not that activated by ceramide, lipopolysaccharides, or phorbol ester. *J Immunol*, 168(6), 2644-51 (2002)

72. J. L. Harousseau: Thalidomide in multiple myeloma: past, present and future. *Future Oncol*, 2(5), 577-89 (2006)

73. P. Zabel, F. U. Schade and M. Schlaak: Inhibition of endogenous TNF formation by pentoxifylline. *Immunobiology*, 187(3-5), 447-63 (1993)

74. P. M. Steed, M. G. Tansey, J. Zalevsky, E. A. Zhukovsky, J. R. Desjarlais, D. E. Szymkowski, C. Abbott, D. Carmichael, C. Chan, L. Cherry, P. Cheung, A. J. Chirino, H. H. Chung, S. K. Doberstein, A. Eivazi, A. V. Filikov, S. X. Gao, R. S. Hubert, M. Hwang, L. Hyun, S. Kashi, A. Kim, E. Kim, J. Kung, S. P. Martinez, U. S. Muchhal, D. H. Nguyen, C. O'Brien, D. O'Keefe, K. Singer, O. Vafa, J. Vielmetter, S. C. Yoder and B. I. Dahiyat: Inactivation of TNF signaling by rationally designed dominant-negative TNF variants. *Science*, 301(5641), 1895-8 (2003)

75. M. M. He, A. S. Smith, J. D. Oslob, W. M. Flanagan, A. C. Braisted, A. Whitty, M. T. Cancilla, J. Wang, A. A. Lugovskoy, J. C. Yoburn, A. D. Fung, G. Farrington, J. K. Eldredge, E. S. Day, L. A. Cruz, T. G. Cachero, S. K. Miller, J. E. Friedman, I. C. Choong and B. C. Cunningham: Small-molecule inhibition of TNF-alpha. *Science*, 310(5750), 1022-5 (2005)

76. C. Wang, G. B. Schuller Levis, E. B. Lee, W. R. Levis, D. W. Lee, B. S. Kim, S. Y. Park and E. Park: Platycodin D and D3 isolated from the root of Platycodon grandiflorum modulate the production of nitric oxide and secretion of TNF-alpha in activated RAW 264.7 cells. *Int Immunopharmacol*, 4(8), 1039-49 (2004)

77. T. L. Wadsworth, T. L. McDonald and D. R. Koop: Effects of Ginkgo biloba extract (EGb 761) and quercetin on lipopolysaccharide-induced signaling pathways involved in the release of tumor necrosis factor-alpha. *Biochem Pharmacol*, 62(7), 963-74 (2001)

78. K. C. Verhoeckx, H. A. Korthout, A. P. van Meeteren-Kreikamp, K. A. Ehlert, M. Wang, J. van der Greef, R. J. Rodenburg and R. F. Witkamp: Unheated Cannabis sativa extracts and its major compound THC-acid have potential immuno-modulating properties not mediated by CB1 and CB2 receptor coupled pathways. *Int Immunopharmacol*, 6(4), 656-65 (2006)

79. S. Shishodia, H. M. Amin, R. Lai and B. B. Aggarwal: Curcumin (diferuloylmethane) inhibits constitutive NF-kappaB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma. Biochem Pharmacol, 70(5), 700-13 (2005)

80. E. Ramiro, A. Franch, C. Castellote, F. Perez-Cano, J. Permanyer, M. Izquierdo-Pulido and M. Castell: Flavonoids from Theobroma cacao down-regulate inflammatory mediators. *J Agric Food Chem*, 53(22), 8506-11 (2005)

81. Z. Qiu, K. Jones, M. Wylie, Q. Jia and S. Orndorff: Modified Aloe barbadensis polysaccharide with immunoregulatory activity. Planta Med, 66(2), 152-6 (2000)

82. C. Punzon, A. Alcaide and M. Fresno: In vitro anti-inflammatory activity of Phlebodium decumanum. Modulation of tumor necrosis factor and soluble TNF receptors. Int Immunopharmacol, 3(9), 1293-9 (2003)

83. J. Piscoya, Z. Rodriguez, S. A. Bustamante, N. N. Okuhama, M. J. Miller and M. Sandoval: Efficacy and safety of freeze-dried cat's claw in osteoarthritis of the knee: mechanisms of action of the species Uncaria guianensis. Inflamm Res, 50(9), 442-8 (2001)

84. A. Perrone, A. Plaza, S. F. Ercolino, A. I. Hamed, L. Parente, C. Pizza and S. Piacente: 14,15-secopregnane derivatives from the leaves of Solenostemma argel. *J Nat Prod*, 69(1), 50-4 (2006)

85. E. A. Ojo-Amaize, P. Kapahi, V. N. Kakkanaiah, T. Takahashi, T. Shalom-Barak, H. B. Cottam, A. A. Adesomoju, E. J. Nchekwube, O. A. Oyemade, M. Karin and J. I. Okogun: Hypoestoxide, a novel anti-inflammatory natural diterpene, inhibits the activity of IkappaB kinase. *Cell Immunol*, 209(2), 149-57 (2001)

86. Z. G. Nie, S. Y. Peng and W. J. Wang: [Effects of ginkgolide B on lipopolysaccharide-induced TNFalpha production in mouse peritoneal macrophages and NF-kappaB activation in rat pleural polymorphonuclear leukocytes]. *Yao Xue Xue Bao*, 39(6), 415-8 (2004)

87. K. Matsunaga, T. W. Klein, H. Friedman and Y. Yamamoto: Epigallocatechin gallate, a potential immunomodulatory agent of tea components, diminishes cigarette smoke condensate-induced suppression of anti-Legionella pneumophila activity and cytokine responses of alveolar macrophages. *Clin Diagn Lab Immunol*, 9(4), 864-71 (2002)

88. H. Matsuda, S. Tewtrakul, T. Morikawa, A. Nakamura and M. Yoshikawa: Anti-allergic principles from Thai zedoary: structural requirements of curcuminoids for inhibition of degranulation and effect on the release of TNF-alpha and IL-4 in RBL-2H3 cells. *Bioorg Med Chem*, 12(22), 5891-8 (2004)

89. H. Matsuda, T. Morikawa, H. Managi and M. Yoshikawa: Antiallergic principles from Alpinia galanga: structural requirements of phenylpropanoids for inhibition of degranulation and release of TNF-alpha and IL-4 in RBL-2H3 cells. *Bioorg Med Chem Lett*, 13(19), 3197-202 (2003)

90. A. Makris, C. E. Thornton, B. Xu and A. Hennessy: Garlic increases IL-10 and inhibits TNFalpha and IL-6 production in endotoxin-stimulated human placental explants. *Placenta*, 26(10), 828-34 (2005)

91. L. Liu, Z. Q. Ning, S. Shan, K. Zhang, T. Deng, X. P. Lu and Y. Y. Cheng: Phthalide Lactones from Ligusticum chuanxiong inhibit lipopolysaccharide-induced TNF-alpha production and TNF-alpha-mediated NF-kappaB Activation. *Planta Med*, 71(9), 808-13 (2005)

92. A. H. Lin, S. X. Fang, J. G. Fang, G. Du and Y. H. Liu: [Studies on anti-endotoxin activity of F022 from Radix Isatidis]. *Zhongguo Zhong Yao Za Zhi*, 27(6), 439-42 (2002)

93. S. H. Lee, G. S. Seo and D. H. Sohn: Inhibition of lipopolysaccharide-induced expression of inducible nitric oxide synthase by butein in RAW 264.7 cells. *Biochem Biophys Res Commun*, 323(1), 125-32 (2004)

94. J. H. Lee, H. S. Jung, P. M. Giang, X. Jin, S. Lee, P. T. Son, D. Lee, Y. S. Hong, K. Lee and J. J. Lee: Blockade of nuclear factor-kappaB signaling pathway and anti-inflammatory activity of cardamomin, a

chalcone analog from Alpinia conchigera. *J Pharmacol Exp Ther*, 316(1), 271-8 (2006)

95. Y. C. Kuo, W. J. Tsai, H. C. Meng, W. P. Chen, L. Y. Yang and C. Y. Lin: Immune reponses in human mesangial cells regulated by emodin from Polygonum hypoleucum Ohwi. *Life Sci*, 68(11), 1271-86 (2001)

96. H. M. Kim, H. Y. Shin, K. H. Lim, S. T. Ryu, T. Y. Shin, H. J. Chae, H. R. Kim, Y. S. Lyu, N. H. An and K. S. Lim: Taraxacum officinale inhibits tumor necrosis factor-alpha production from rat astrocytes. *Immunopharmacol Immunotoxicol*, 22(3), 519-30 (2000)

97. H. M. Kim, E. H. Lee, H. J. Na, S. B. Lee, T. Y. Shin, Y. S. Lyu, N. S. Kim and S. Nomura: Effect of Polygala tenuifolia root extract on the tumor necrosis factor-alpha secretion from mouse astrocytes. *J Ethnopharmacol*, 61(3), 201-8 (1998)

98. H. Kim, E. Lee, T. Lim, J. Jung and Y. Lyu: Inhibitory effect of Asparagus cochinchinensis on tumor necrosis factor-alpha secretion from astrocytes. *Int J Immunopharmacol*, 20(4-5), 153-62 (1998)

99. A. K. Kiemer, T. Hartung, C. Huber and A. M. Vollmar: Phyllanthus amarus has anti-inflammatory potential by inhibition of iNOS, COX-2, and cytokines via the NF-kappaB pathway. *J Hepatol*, 38(3), 289-97 (2003)

100. O. H. Kang, Y. A. Choi, H. J. Park, J. Y. Lee, D. K. Kim, S. C. Choi, T. H. Kim, Y. H. Nah, K. J. Yun, S. J. Choi, Y. H. Kim, K. H. Bae and Y. M. Lee: Inhibition of trypsin-induced mast cell activation by water fraction of Lonicera japonica. *Arch Pharm Res*, 27(11), 1141-6 (2004)

101. H. K. Ju, T. C. Moon, E. Lee, S. H. Baek, R. B. An, K. Bae, K. H. Son, H. P. Kim, S. S. Kang, S. H. Lee, J. K. Son and H. W. Chang: Inhibitory effects of a new iridoid, patridoid II and its isomers, on nitric oxide and TNF-alpha production in cultured murine macrophages. *Planta Med*, 69(10), 950-3 (2003)

102. H. Z. Jin, D. Lee, J. H. Lee, K. Lee, Y. S. Hong, D. H. Choung, Y. H. Kim and J. J. Lee: New sesquiterpene dimers from Inula britannica inhibit NF-kappaB activation and NO and TNF-alpha production in LPS-stimulated RAW264.7 cells. *Planta Med*, 72(1), 40-5 (2006)

103. R. Ishii, M. Horie, K. Saito, M. Arisawa and S. Kitanaka: Inhibition of lipopolysaccharide-induced pro-inflammatory cytokine expression via suppression of nuclear factor-kappaB activation by Mallotus japonicus phloroglucinol derivatives. *Biochim Biophys Acta*, 1620(1-3), 108-18 (2003)

104. K. F. Hua, H. Y. Hsu, Y. C. Su, I. F. Lin, S. S. Yang, Y. M. Chen and L. K. Chao: Study on the antiinflammatory activity of methanol extract from seagrass Zostera japonica. *J Agric Food Chem*, 54(2), 306-11 (2006)

105. R. Grzanna, P. Phan, A. Polotsky, L. Lindmark and C. G. Frondoza: Ginger extract inhibits beta-amyloid peptide-induced cytokine and chemokine expression in cultured THP-1 monocytes. *J Altern Complement Med*, 10(6), 1009-13 (2004)

106. M. Gao, J. Zhang and G. Liu: Effect of diphenyl dimethyl bicarboxylate on concanavalin A-induced liver injury in mice. *Liver Int*, 25(4), 904-12 (2005)

107. D. Duansak, J. Somboonwong and S. Patumraj: Effects of Aloe vera on leukocyte adhesion and TNF-alpha and IL-6 levels in burn wounded rats. *Clin Hemorheol Microcirc*, 29(3-4), 239-46 (2003)

108. K. S. Chun, J. Y. Kang, O. H. Kim, H. Kang and Y. J. Surh: Effects of yakuchinone A and yakuchinone B on the phorbol ester-induced expression of COX-2 and iNOS and activation of NF-kappaB in mouse skin. *J Environ Pathol Toxicol Oncol*, 21(2), 131-9 (2002)

109. J. Y. Cho, J. Park, E. S. Yoo, K. Yoshikawa, K. U. Baik, J. Lee and M. H. Park: Inhibitory effect of lignans from the rhizomes of Coptis japonica var. dissecta on tumor necrosis factor-alpha production in lipopolysaccharide-stimulated RAW264.7 cells. *Arch Pharm Res*, 21(1), 12-6 (1998)

110. M. M. Chan: Inhibition of tumor necrosis factor by curcumin, a phytochemical. *Biochem Pharmacol*, 49(11), 1551-6 (1995)

111. X. L. Bi, J. Y. Yang, Y. X. Dong, J. M. Wang, Y. H. Cui, T. Ikeshima, Y. Q. Zhao and C. F. Wu: Resveratrol inhibits nitric oxide and TNF-alpha production by lipopolysaccharide-activated microglia. *Int Immunopharmacol*, 5(1), 185-93 (2005)

112. H. S. Ban, K. Suzuki, S. S. Lim, S. H. Jung, S. Lee, J. Ji, H. S. Lee, Y. S. Lee, K. H. Shin and K. Ohuchi: Inhibition of lipopolysaccharide-induced expression of inducible nitric oxide synthase and tumor necrosis factor-alpha by 2'-hydroxychalcone derivatives in RAW 264.7 cells. *Biochem Pharmacol*, 67(8), 1549-57 (2004)

113. H. Abuarqoub, R. Foresti, C. J. Green and R. Motterlini: Heme oxygenase-1 mediates the anti-inflammatory actions of 2'-hydroxychalcone in RAW 264.7 murine macrophages. *Am J Physiol Cell Physiol*, 290(4), C1092-9 (2006)

114. M. J. Abad, P. Bermejo, M. Alvarez, J. A. Guerra, A. M. Silvan and A. M. Villar: Flavonoids and a sesquiterpene lactone from Tanacetum microphyllum inhibit anti-inflammatory mediators in LPS-stimulated mouse peritoneal macrophages. *Planta Med*, 70(1), 34-8 (2004)

Abbreviations: anti-TNF: anti-tumor necrosis factor; API-2: Akt/protein kinase B signaling inhibitor-2; CD: Crohn's disease; c-IAP-1: inhibitor of apoptosis-1; COX-2, cyclooxygenase-2; ERK-1/2: extracellular signal-regulated kinase-1/2; FADD: Fas-associated death domain; FLICE:

FADD-like ICE; JNK: c-Jun NH(2)-terminal kinase; IkappaB, inhibitory subunit of NF-kappaB; IKK: IkappaBalpha kinase; iNOS: inducible nitric oxide synthase; LOX-2: lipoxygenases-2; MAPK: mitogen-activated protein kinases; MMP-9, matrix metalloproteinase-9; NIK: NF-kappaB inducing kinase; NF-kappaB: nuclear factor kappa B; TNF: tumor necrosis factor; RANKL: receptor activator of NF-kappaB ligand; TACE: TNF-activating converting enzyme; TRADD: TNF receptor-associated death domain; TRAF2:, TNF receptor-associated factor; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand.

Key Words TNF, Inflammation, Cancer, Natural Products, Cytokine, Tumor Necrosis Factor, Review

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