

Developmental Phases of Inflammation-Induced Massive Lymphoid Hyperplasia and Extensive Changes in Epithelium in an Experimental Model of Allergy

Implications for a Direct Link Between Inflammation and Carcinogenesis

Mahin Khatami*

Direct evidence that inflammation is linked to carcinogenesis has yet to be established. Very few data are available on the developmental phases of inflammation-induced immune dysfunction that may lead to tumorigenesis. In a series of studies conducted in the 1980s and 1990s, an experimental model of acute and chronic inflammation was established in guinea pig conjunctiva by topical application of fluoresceinyl ovalbumin (FLOA) for up to 30 months. In this updated report, some of the findings are reanalyzed and expanded to identify that at least 3 developmental phases were involved during the entire course of inflammatory responses including (1) an acute response (phase A) involving IgE-mast cell sensitization and degranulation; (2) an intermediate phase (phase B), a desensitization phenomenon and loss of mast cell function and neovascularization; (3) a chronic response (phase C) and induction of massive lymphoid hyperplasia, follicular formation with germinal centers, increased swollen goblet cells, extensive epithelial thickening and thinning, and angiogenesis. The results suggest evidence of a direct association between inflammation and the development of tumor-like lesions in lymphoid tissues and extensive changes in adjacent epithelia. Confirmation that inflammation induces irreversible changes in lymphoid and epithelial tissues leading to lymphoid tumorigenesis and/or carcinogenesis requires further studies. Understanding the developmental phases of immune dysfunction may provide unique opportunities for diagnosis and treatment of inflammatory diseases, autoimmune disorders, and cancers including lymphomas associated with Sjögren syndrome, squamous cell carcinoma of the conjunctiva, and other lymphomas or epithelial cancers. It is suggested that inflammatory mediators are ideal targets (biomarkers) for diagnosis, chemoprevention, and therapy for several cancers.

Keywords: inflammation, cancer, allergy, lymphoid hyperplasia, mast cells, macrophages, goblet cells, immune dysfunction

University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

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**Address for correspondence: The National Cancer Institute, Division of Cancer Prevention, National Institutes of Health, 3151 EPN, 1630 Executive Plaza, Bethesda, MD 20892. E-mail: Khatamim@mail.nih.gov*

INTRODUCTION

Inflammation is a series of complex immunologic, genetic, and physiologic responses of tissues toward injuries, irritants, allergens, infections, other environmental hazards, or carcinogens. The dysfunction of immune and nonimmune systems, leading to the development of abnormal cell growth patterns and progression to malignancies, are very likely the result of extensive slow and progressive alterations in the balance between cellular and humoral responses in the course of repeated tissue irritations causing chronic and persistent inflammatory conditions.

Direct evidence that persistent and recurrent inflammation is associated with the genesis of benign or malignant tumors has yet to be established. However, extensive circumstantial evidence supports an association between inflammation and several cancer sites including the colon, esophagus, stomach, liver, breast, pancreas, prostate, bladder, ovary, and lung.¹⁻⁵ In addition, asthmatic patients have been reported to have an increased risk of developing lung and other cancers⁶ and higher mortality rates.⁷

Very few data are currently available on the developmental phases of inflammation-induced immune dysfunction that may lead to carcinogenesis. A reason that no direct evidence of an association between inflammation and tumorigenesis has been established is that few studies have been designed to demonstrate the systematic, dynamic, and progressive changes of inflammatory responses, including participation of acute inflammatory pathways in the initiation of immune dysfunction during persistent inflammatory conditions. Perhaps a fundamental question that needs to be further addressed is how the growth-arresting properties of acute inflammation that is inherent in the body's immune defense mechanisms against infections, chemical insults, carcinogens, and injuries switch to growth-promoting functions under persistent inflammatory conditions that could lead to development of cancer.

Among the organ systems, lung airways and ocular conjunctiva represent ideal tissues to demonstrate a wide range of inflammatory diseases in humans. Both lung and eyes are among the organs that directly encounter environmental allergens and hazards that often cause various allergies. The clinical and immunologic features of allergic reactions in the lung such as asthma or emphysema⁶⁻⁸ may be comparable with ocular allergic conditions such as vernal, kerato-, and atopic conjunctivitis and hay fever.⁹⁻¹¹ Furthermore, the principal immune response mechanisms involved in the induction of allergies and cancers in the lung^{5,8,12,13} appear comparable with those occurring in the ocular allergies,⁹⁻¹¹ squamous cell carcinomas of the conjunctiva in patients with atopic eczema,¹⁴ and lymphomas associated with Sjögren syndrome.¹⁵

To better understand the molecular and immunologic basis of inflammatory responses in allergic diseases and the relationship between inflammation and the development of hyperplasia of lymphoid tissues and extensive changes in epithelia, experimental models of human acute and chronic inflammatory and infectious diseases were established using conjunctival tissue.^{11,16-27} In this article, data selected from a series of studies of models of acute and chronic inflammation are reanalyzed and expanded to identify the developmental phases of immune responses during the

entire course of inflammation. Furthermore, the intimate role of blood components and vascular function in all phases of inflammatory responses is demonstrated.

EXPERIMENTAL PROCEDURES

Acute and chronic inflammation in conjunctival tissue was induced in guinea pigs, monkeys, and dogs as previously described.^{11,16-27} For the majority of studies described in this article, ocular tissue of the guinea pigs was sensitized and challenged by repeated topical applications of the antigen (ovalbumin, conjugated with fluorescein, FLOA), 2 to 4 times per day for the first 1½ weeks and 2 to 6 times per month for up to 30 months. Eyes were sensitized unilaterally or bilaterally, with or without application of *Ascaris suum* adult extracts, active systemic infection with *A. suum* larvae and eggs.^{11,16-27} Organ and tissue cultures from sensitized conjunctiva and spleen were prepared to examine antibody profiles as previously described.^{21,23} In a series of experiments, the eyes of newborn babies from sensitized pregnant animals were topically challenged only once or twice by FLOA within 3 weeks after the birth. In another series of experiments, the eyes were topically treated with a mixture of antigen and tumor-promoting agents (TPAs, phorbol esters) for a period of up to 6 months.^{26,27}

Inflammatory responses were monitored clinically, histopathologically, and/or by immunologic assays. Sera or culture media from sensitized animals were tested for the presence of homocytotropic IgE antibodies or IgG1, IgG2, IgM, and IgA antibodies as previously described.^{11,16-25} Histamine and prostaglandins were measured by radioimmunoassays or ELISA. Mast cells, eosinophils, basophils, goblet cells, macrophages, and vascular leakage were monitored by compound 48/80, a mast cell-degranulating agent, histologic procedures, or fluorescent microscopy leakage experiments.^{11,16-25}

RESULTS AND ANALYSES OF DATA

Clinical and histopathologic findings at various time points in the inflammatory process demonstrated that there were at least 3 distinct developmental phases of inflammatory responses during the entire course of sensitization and challenge, including (1) induction of acute type 1 hypersensitivity reactions (phase A); (2) down-regulation of acute responses and exhaustion/loss of functional mast cells (phase B, intermediate phase), and (3) chronic inflammation-induced massive hyperplasia of lymphoid tissues and extensive pathologic changes in the epithelia (phase C)^{11,16,20-27} (Table 1).

Phase A (early/acute responses and immediate hypersensitivity type 1 reactions: mast-cell activation and degranulation)

The clinical responses that began about 9–12 days after repeated topical sensitization with FLOA were maximized in some animals for up to 25 days.^{11,16} Prominent clinical responses included conjunctiva and lid edema, scratching of the lid surface, and tear production, which was often accompanied by a milky (fatty acid) secretion in the tear fluids. The clinical reactions that started within the first few minutes (7–20 minutes) after antigen challenge subsided within the first hour after the challenge and the eyes appeared normal.^{11,16} Newborn babies (2–3 weeks after birth) of repeatedly sensitized and challenged pregnant guinea pigs responded strongly to the first or second topical challenge with antigen (data not shown). No prior stimulation or sensitization period (9–12 days' duration) was required for producing strong type 1 reactions, suggesting fetus mast-cell sensitization through maternal antibody transfer.

As schematically shown in Figure 1, the net effects of acute inflammatory responses are antigen removal from the epithelial tissue through activation of mast cells and other cellular and humoral responses. Mast-cell degranulation was accompanied by the release of histamine followed by vascular hyperpermeability resulting in efflux of plasma proteins and infiltration of inflammatory cells into the tissue. Within 1 hour after a strong acute response, tissues appeared normal and quiescent.^{11,16} These acute response pathways may be considered reversible (Table 1).

Histopathologic findings included mast cell degranulation, infiltration of eosinophils and neutrophils into the conjunctival tissues and vascular hyperpermeability or increased vascular leakage^{11,16,20–27} (Table 1).

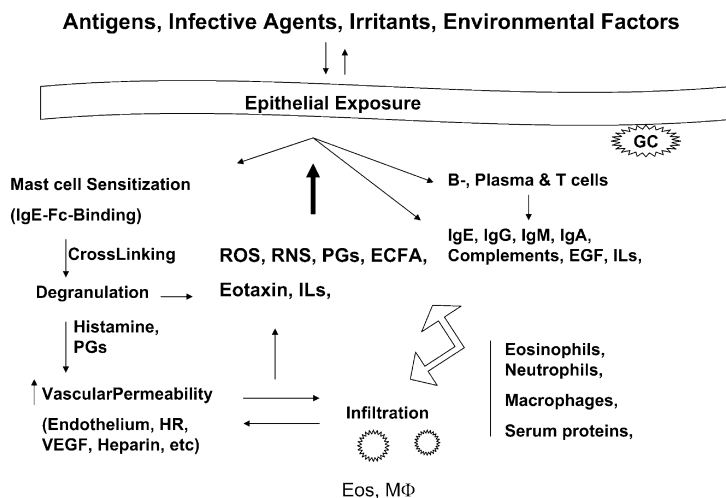
ANTIBODY PRODUCTION

Production of antibodies including homocytotropic IgE and IgG, IgG1, IgM, or IgA from activated B or plasma

Table 1. Developmental phases of inflammatory responses in conjunctival-associated lymphoid tissues.

1. Phase 2. Treatment 3. Duration 4. Comments	Major clinical findings	Major histopathological findings
1. Phase A, Acute 2. Antigen alone 3. Within a month 4. Reversible	Strong type 1 reactions, tearing, mucus and/or milky secretions from vascular permeability	Mast cell degranulation, eosinophil infiltration, increased vascular leakage
1. Phase B, Intermediate 2. Antigen alone 3. Within two months 4. Reversible (?)	Weak type 1 reactions, mucus secretion, tissue appearance beefy red, vascular hyperpermeability and neovascularization	Decreased number of mast cells, extensive eosinophils in mucus secretion, eosinophil in subepithelial tissues, eosinophils in goblet cells, epithelial thinning or thickening, new vessel formation
1. Phase C, Chronic 2. Antigen alone 3. Twelve to 30 months 4. Irreversible (?)	Nodules, lymphofollicular formation, extensive neovascularization, formation of yellowish lipid-like material around and beneath the cornea	Massive hyperplasia of lymphoid tissues, phagocytic macrophages, partially granulated mast cells, plasma cells, lymphatic channels, immunoglobulin stainings, germinal center, immature lymphocytes, swollen goblet cells, extensive epithelial thickening or thinning, extensive neovascularization/angiogenesis
1. Phase C, Chronic (?) 2. Antigen + TPA* 3. Two to 6 months 4. Irreversible (?)	Nodules, lymphofollicular formation, extensive neovascularization	Not determined

*TPA, tumor promoting agents.



reactive nitrogen species (RNS), prostaglandins, eosinophil chemotactic factor of anaphylaxis (ECFA) or Eotaxin, interleukins (ILs). Activation of these pathways will cause removal of irritants from the tissue and tissue repair.

cells or tissues were demonstrated as previously described.^{11,20-23} In some instances, topically sensitized and challenged animals with FLOA alone had no detectable serum IgE antibody, suggesting that excess circulating IgE antibody was not required to produce maximal clinical reactions and mast-cell degranulation.^{11,21-23,25} The synthesized IgE antibody was perhaps capable of efficiently binding to high-affinity Fc receptor molecules on the surface of activated local or other tissue mast cells.^{11,25} Support for this came from several lines of observations. First, unilaterally sensitized and challenged eye produced maximal reactivity to a single topical challenge with antigen in the contralateral untreated eye.^{11,16} Second, newborn guinea pigs, from sensitized pregnant animals, responded strongly after only the first or second challenge by antigen suggesting newborn conjunctival mast-cell sensitization (IgE binding) through maternal circulation (data not shown). In addition, in certain instances, animals with severe ocular responses also produced wheezing and asthma-like episodes^{26,27} suggesting antigen-induced activation of lung mast cells. Other possibilities for sensitization of lung mast cells and asthma-like symptoms may include lung tissue exposure to antigen through nasal routes.

DETECTION OF INFLAMMATORY MEDIATORS

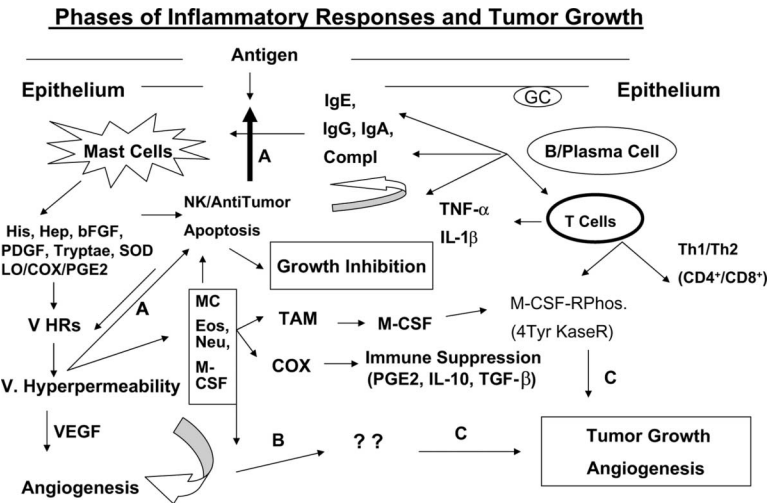
Time-course kinetics of the release of histamine and prostaglandins (eg, prostacyclin, 6-keto-PGF 1α , a stable product of PGI-2) were examined in the tears of sensitized eyes over a 24-hour period.²⁴ Histamine release

FIGURE 1. Phase A: schematic representation of acute inflammatory responses in epithelium. Acute inflammatory response pathways (reversible) include sensitization, maturation, and activation of humoral and cellular systems including mast cells, lymphocytes (B, plasma, and T cells); goblet cells (GC); synthesis of immunoglobulins/antibodies (IgE, IgG, IgM, IgA), epithelial growth factor (EGF), interleukins (ILs), IgE binding to high-affinity mast-cell Fc receptors; degranulation of mast cells; release of preformed or newly synthesized components such as histamine, heparin, and prostaglandins (PGs); increased permeability of the vasculature, endothelial histamine receptors (HR), and vascular endothelial growth factor (VEGF); infiltration of blood neutrophils, eosinophils, macrophages, and plasma proteins; induction of toxic components such as reactive oxygen species (ROS),

was maximal during the first few minutes (10 minutes) after the challenge and returned to baseline thereafter. The 6-keto-PGF 1α release also peaked during the first 10 minutes of challenge but continued for an extended period. The ratio of tear 6-keto-PGF 1α to histamine increased more than 16-fold over the 2 hours after antigen challenge.²⁴ This suggested that in the acute phase of responses, histamine acted as a primary inflammatory mediator stimulating the secondary production and release of prostanoid by constitutive (eg, vascular) and/or infiltrating inflammatory cells during the responses.²⁴ The possibility of the synthesis and release of prostaglandins during mast cell degranulation in phase A has also been suggested (Figs. 1-3).¹¹ Whether the time-course kinetics, composition, or ratios of prostaglandins to histamine would alter, eg, favoring PGE 2 production and immune suppression, during the course of immune responses remains to be studied.

Phase B (intermediate phase, down-regulation of type 1 inflammatory responses: mast-cell desensitization, loss/exhaustion of mast cells, and neovascularization)

Two response profiles were identified in the intermediate phase. Continuous topical challenges with antigen in sensitized animals that initially produced strong type 1 reactions induced an exponential decrease in the extent of responses within 2 months of repeated challenges, as examined clinically and histopathologically^{11,16} (Table 1). Papillary irregularities of palpebral conjunctival surface and tenacious secretion were also noted in some animals.¹¹ Conjunctival tissue appeared beefy red,¹¹ and the appearance of eyes did not completely return to normal, with regard to changes in the



(Neu), eosinophils (Eos), and macrophages (MΦ), macrophage colony-stimulating factor (M-CSF), vascular endothelial growth factor (VEGF), cell adhesion molecules (CAM), goblet cells (GC), tumor necrosis factor α (TNF- α), interleukins (ILs), etc., resulting in apoptosis/growth arrest and tissue repair. Intermediate phase (B) responses initiate pathways with impaired mast cell functions to produce cytokine signals by inflammatory cells (eg, mast cells and MΦ) with pleiotropic functions, favoring synthesis of abnormal growth factors; M-CSF receptor phosphorylation (M-CSF-RPhos), immune suppressor factors (eg, COX and PGE2), ILs (IL-10), and transforming growth factor β (TGF- β); activation of oncogene and growth promotion leading to chronic phase (C) responses, which could be irreversible pathways that favor tumor growth.

vasculature, within 1 or 2 hours after the acute responses subsided. This suggested initiation of neovascularization (angiogenesis) of the conjunctival tissue at the intermediate phase.

Two possibilities for the desensitization phenomenon were considered¹⁶: (1) the loss of number of functional mast cells and (2) the loss of target organ sensitivity (eg,

vascular endothelial histamine receptors) to mast-cell mediators.

These possibilities were separately examined. Clinically desensitized tissue did not respond, or responded minimally, to topical treatment with compound 48/80. However, the retained response to topical histamine indicated that the progressive loss of acute/type-1 reactivity was due to a loss or exhaustion of functional/mature mast cells and not to changes in histamine receptors. Histologic examination of mast cells showed that the density of granulated intact mast cells was inversely proportional to the number of challenges with antigen or compound 48/80.¹⁶ It was concluded that the induction and down-regulation of immediate hypersensitivity reactions were primarily regulated by the extent of mast-cell degranulation and function.¹⁶

Another profile of inflammatory responses was noted in the persistently reactive tissues that produced moderate immediate type 1 hypersensitivity reactions beyond 25 days. Prominent histopathologic features in tissue that was persistently and moderately responsive to antigen challenge included extensive eosinophil infiltrations in the epithelia, conjunctival papillary changes, epithelial thickening or thinning, numerous goblet cells infiltrated with eosinophils, subepithelial lymphoid cell infiltrates, and new vessel formation.^{11,16} Eosinophil infiltration was much more extensive in the sensitized tissue that was reactive within the 2 months of repeated stimulation compared with the acute reactions at phase A. Furthermore, combination of systemic

Mast Cells and Related Inflammatory Mediators

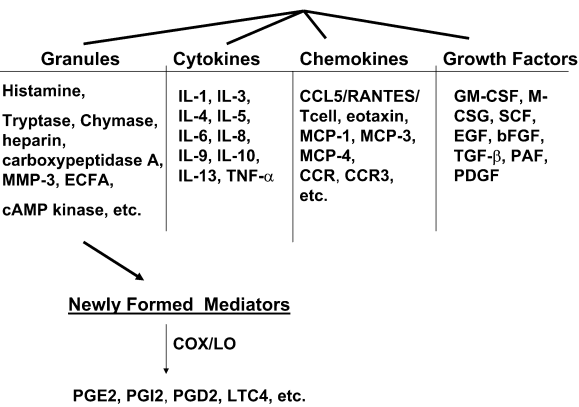


FIGURE 3. Mediators released from mast cell granules, cytokines, chemokines, and growth factors synthesized and released from activated mast cells and neutrophils involved in immunity against tumor include interleukins (ILs) and interferons (IFNs), granulocyte colony-stimulating factors (G-CSF), tumor necrosis factor α (TNF- α), etc. These neutrophil-associated factors quickly recruit a massive local reaction against tumor cells.

FIGURE 2. Schematic representation of developmental phases of inflammatory responses leading to tumor growth promotion pathways. Acute phase (A) response pathways (reversible) involve sensitization and activation of local and systemic blood components and release of toxic mediators to remove antigen. These include B, plasma, and T cells (Th1, Th2, CD4⁺, CD8⁺), and mast cells; synthesis of antibodies (IgE, IgG, IgA), complement (Compl) proteins; degranulation of mast cells; release of preformed or newly synthesized components such as histamine (His), heparin (Hep), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), tryptase, cyclooxygenase (COX) pathways, prostaglandins (eg, PGI2, PGE2), and superoxide dismutase (SOD); infiltration of neutrophils

infection with *A. suum* and topical application with FLOA produced the highest percentage of eosinophil infiltration into the mucus secretion.¹¹

Phase C (chronic inflammation-induced development of massive hyperplasia of conjunctiva-associated lymphoid tissues and significant changes in epithelia)

Clinical and immunopathologic findings in animals that were continuously challenged with FLOA for 12–30 months produced a variety of responses ranging from papillary changes, neovascularization (angiogenesis), development of massive hyperplasia of lymphoid tissues, and extensive thickening and/or thinning of the epithelia²² (Table 1). Weak or minimal acute reactions were produced upon challenge with antigen, even when eyes were rested for a period of 2 weeks between challenges. In some animals, small various-size nodules and tumor-like discrete rounded and elevated follicles developed and protruded from the lower and upper palpebral fornix conjunctiva and at the caruncle²² independent of antigen challenge (eg, during the rest period) (Khatami, unpublished observations). In some enlarged follicles, the rich subepithelial vascular network disappeared centrally, resulting in the appearance of discrete nodules that were encircled by a ring of new blood vessels.²² In other instances, the surface of enlarged follicular masses (nodules) showed various-size discrete follicles progressing from the small follicles with an overlying arborizing network of vessels beneath the conjunctival epithelium, to the larger follicles typically free of a central but surrounded by a peripheral vascular net.²² In some animals, a yellowish lipid-like material appeared often beneath and around the corneal surface (Table 1) (Khatami, unpublished observations). The histopathologic features and composition of the yellowish material remain unknown. It is possible that persistent inflammation caused gradual accumulation and deposition of blood-derived proteins or lipid-like material and/or mucinous components from activated goblet cells that did not clear from the tissues.

Histopathologic findings of the lymphoid tissues included hyperplastic lymphoid nodules composed of a collection of densely packed small lymphocytes with scanty cytoplasm, darkly stained peripheral nuclear chromatin with many moderate nuclear border irregularities and indentations, and prominent nucleoli²² (Table 1, Fig. 4). The germinal centers contained various-size lymphoid cells with larger, paler nuclei and more abundant cytoplasm.²² The cross-sectional area of the massive hyperplastic lymphoid nodules from the animals that were continuously challenged

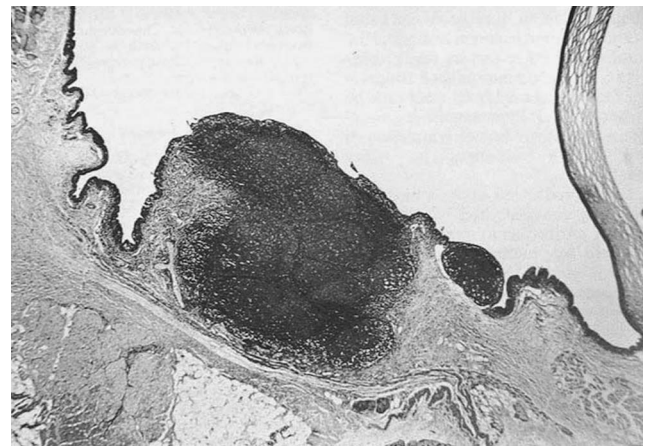


FIGURE 4. Histopathologic section of eyelid of repeatedly topically immunized and challenged guinea pig shows massive hyperplasia of conjunctiva-associated lymphoid tissue (7- μ m paraffin section, Luna's eosinophil stain, $\times 24$). From Khatami et al.²² Copyright American Medical Association © 1989. All rights reserved.

with antigen was at least 5 times greater than the lymphoid tissues in normal untreated animals (Fig. 4).²² The presence of prominent follicle formation and massive hyperplasia of lymphoid tissue were found in some animals after 12–16 months and in others after 30 months of continued stimulation with antigen. The lymphoid cells, which in normal conjunctiva were confined peripherally within a thin fibrous capsule, appeared to have lost the integrity of fibrous capsule in hyperplastic tissues. In the hyperplastic tissues, small lymphocytes extended beyond the fibrous capsule into the surrounding conjunctival substantia propria.²² Numerous large phagocytic macrophages that contained large and small dark-staining, round or vacuolated inclusions and scattered plasmacytoid or plasma cells also were present in the germinal centers. The germinal centers were separated from the surrounding small lymphocytes in many regions by an irregular basement membrane-like capsule.²² The follicles were composed predominantly of lymphocytes, scattered activated histiocytes (phagocytic macrophages), small numbers of plasma cells, and partially degranulated mast cells.²² Abnormal thinning and thickening of epithelial cells were often noted within the same tissue section²² (Table 1, Fig. 4).

Numerous engorged, swollen mucus-secreting goblet cells were present within the epithelial layer.²² These cells appeared to discharge their contents onto the conjunctival epithelial surface. In addition, partially degranulated mast cells were present immediately beneath the conjunctival epithelium and scattered through the lymphoid tissue.²² The immunoperoxidase assays of frozen

sections of hyperplastic lymphoid tissue showed dense collections of IgG- and IgA-positive cells detected in the B-lymphocyte areas of the germinal centers.²²

In a series of experiments, when antigen was mixed with TPAs (phorbol esters), the time course for development of massive lymphoid hyperplasia of the conjunctival tissues shifted within 2–6 months of topical challenges compared with FLOA challenge alone.^{26,27} It was suggested that TPAs additionally promoted tissue growth through activation of protein kinase C^{26,27} (Table 1).

The production of IgE, IgG1, IgG2, IgM, and IgA antibodies were measured in organ cultures of hyperplastic lymphoid tissue or splenic cells or sera obtained from animals that were sensitized for up to 30 months.^{11,21–23,25} Assays for IgE antibody production were negative, perhaps due to high-affinity IgE binding to Fc receptor on mast cells. The ratios of IgG1:IgG2 antibodies produced by single cell cultures of lymphoid tissue and splenic cells were 15.0 and 3.0, respectively. Also, the serum levels of total anti-FLOA IgG antibody were 100 times greater than cell culture media.^{21,22}

It should be emphasized that the development of tumor-like lesions in the lymphoid tissues occurred often in animals that showed minimal early type 1 responses toward antigen challenges from the onset of induction of inflammation.²²

DISCUSSION

In an inflammatory state, the nature (quality), quantity, frequency, duration (extent) of tissue exposure to irritants or infective agents, and the profiles of immune responses are believed to determine the outcome of the inflammatory response, ie, whether to support cell growth inhibition/arrest (apoptosis) or growth promotion, proliferation, or tumorigenesis (Fig. 2). The balance between the mediators produced and released for growth-arrest purposes and those synthesized and released for growth promotion and proliferation may be significantly altered or shifted during the developmental phases of long-standing inflammatory processes (Fig. 2).

Blood and tissue elements, originating from stem cells, such as the mast cells, eosinophils, macrophages, and dendritic cells, are the key response components in the defense against foreign agents (eg, allergens/antigens, infective agents, carcinogens) as well as tumor surveillance. These components are capable of generating large amounts of highly toxic and reactive molecules such as reactive oxygen species (ROS), reactive nitrogen species (RNS), eosinophil chemotactic factor of anaphylaxis (ECFA), and a number of proteases and

lipid products to remove the irritants or reject the tumors^{11,28,29} (Figs. 1–3). Blood polymorphonuclear neutrophils (PMNs) or leukocytes have been identified as the key elements actively engaged in a complex cross-talk with the immune system and vascular endothelial cells that bridges the innate and adaptive immunity.^{28,29} In cooperation with T lymphocytes, PMNs produce large numbers of cytokines and chemokines to reject tumor cells.²⁸

The role of homocytotropic IgE binding to Fc receptor mast cells in mediating allergic responses have been extensively studied.^{11,16,25,30–33} The net effect of a strong or weak acute response due to functionality of mast cells and IgE–Fc receptor cross-linking properties on the permeability of irritants to interepithelial and subepithelial cells may be significantly different (Fig. 2).²² A strong type 1 reaction may restrict the penetration of antigen to the transepithelial surfaces by an outward flow of fluids (eg, tearing and/or mucus secretion) or copious leakage of vascular blood components.^{11,22} On the other hand, a weak initial type 1 response may have a minimal antigen-clearing effect due to the presence of immature mast cells or alterations in vascular histamine receptors that could lead to inefficient infiltration of inflammatory cells or plasma. The results will be a greater net promotion of antigen penetration due to increased epithelial exposure to higher levels of antigen.^{16,22}

Mast cells have been shown to directly and indirectly influence cellular growth and function of eosinophils, macrophages or T cells, T cell-derived cytokines (eg, IL-5), neutrophil-derived serine proteases, and vascular function^{11,16,30–33} (Figs. 2 and 3). In our studies, the relative number of functional (mature) or fully granulated mast cells decreased during intermediate and chronic phases of inflammation.^{16,22} It is possible that fully granulated mast cells possess tumoricidal properties while partially granulated or “leaky” mast cells have tumor-growth-promoting and angiogenic function. Others have reported a decrease in the ratios of mast cells to tumor cells in patients with pulmonary adenocarcinoma, which correlated with a decrease in survival rates.³¹

Macrophage colony-stimulating factor (M-CSF) is a lymphohematopoietic cytokine that has pleiotropic properties for differentiation of monocyte precursors, tumor arrest, as well as tumor growth^{28,34,35} (Fig. 2). In our studies, the presence of numerous histiocytes and vacuolated macrophages and increased new vessel formation were among the prominent features in lymphoid hyperplasia and epithelial lesions.²² Suzuki et al³⁶ reported that M-CSF was highly sensitive and specific for diagnosis of human ovarian cancers, such as malignant germ cell tumors, squamous cell

carcinoma arising from mature teratoma, immature teratoma, and particularly dysgerminoma of the ovary, as compared with the classic ovarian tumor marker (ie, CA-125). Mroczko et al³⁷ also compared diagnostic values of M-CSF and granulocyte colony-stimulating factor (G-CSF) with known markers such as CEA and CA-19-9 in colorectal cancer patients. The diagnostic specificities of M-CSF and G-CSF were 95%, and the M-CSF predictive value was higher than the predictive value for G-CSF. M-CSF was suggested to be a suitable marker for the early detection of these cancers,^{36,37} particularly the germinal cells of ovarian cancers.³⁶ M-CSF may prove to be an ideal biomarker for detection of several other cancers such as prostate, breast, lung, and colorectal cancers, particularly if it is used in combination with other conventional markers.

While little is understood about the role of mucus-secreting goblet cells in the genesis of tumors, the increased presence of swollen goblet cells in the tumor-like lesions of conjunctiva epithelia²² or perhaps other epithelial tissues may play a role during the developmental phases of inflammatory responses. In our studies, heavy eosinophil infiltration into goblet cells was identified during the intermediate stage (phase B) of inflammatory responses.¹¹ Microglandular goblet cell carcinoma, also known as adenocarcinoid, crypt cell carcinoma, goblet cell carcinoid, mucinous carcinoid, or activated goblet cells have been found in the carcinoid tumors of the appendix, stomach and intestinal cancers, colonic carcinomas, Barrett esophagus, lung cancer, neoplasia of endocrine systems, or appendicitis.³⁸

The immunoregulatory mechanisms responsible for the significant increase in the ratio of IgG1 to IgG2 antibody in hyperplastic lymphoid tissue are not clear. It was suggested that the frequent and large dose penetration of antigen into the substantia propria favor this type of tissue-specific immunoglobulin response.²⁰⁻²³ Chronic and persistent inflammatory responses may alter the expression of immunoglobulins and B-cell memory responses.^{21,22,25} Daly et al²⁹ noted that the diversity of transgenic models for expression of B lymphocytes in CCL2-deficient mice profoundly influence the normal profiles of immunoglobulin subclasses. Others have reported abnormalities in the B-cell memory response in patients with primary Sjögren syndrome (dry eyes), an autoimmune disorder associated with lymphoid follicles¹⁵ and perhaps its progression to lymphoma.

While epithelial tissues are often the primary targets for inflammation-induced tissue damage, the impaired integrity and function of vascular systems perhaps equally or even more importantly contribute to the development of progressive lesions in the tissues (Figs. 1 and 2, Table 1). In our studies, the hyperpermeability

of the vascular system during the acute phase was perhaps a transient phenomenon, ie, as soon as the clinical reactions subsided, the vascular tissues also appeared normal. However, the appearance of neovascularization (angiogenesis) that was initially noted during the intermediate response phase (phase B) progressively increased during the chronic phase (phase C)^{11,16,22} (Table 1, Fig. 2).

The developmental phases of the inflammatory responses in a model of allergic conjunctivitis identified in this article are believed to be the first experimental evidence suggesting a direct link between chronic and persistent inflammation and the development of tumor-like lesions in lymphoid tissue and extensive alterations in epithelia accompanied by angiogenesis. Many of the immunopathologic features described in the model appear to mimic the pathologic findings in several other epithelial and lymphoid tissues in human tumors. Confirmation for inflammation-induced irreversible progressive changes in lymphoid and epithelial tissues and the development of pre-cancer or malignant tumors awaits further studies.

SUMMARY

The clinical and immunopathologic findings in a model of acute and chronic inflammation included at least 3 developmental phases of inflammatory responses leading to the development of tumor-like lesions in lymphoid tissues accompanied by extensive changes in epithelia and angiogenesis. The findings suggested that the profiles of immune responses are dynamic and complex and progressively change the signaling mechanisms in favor of tumor growth promotion. More studies are required to demonstrate the details of humoral and cellular response mechanisms during the developmental phases of inflammation-induced immune dysfunction that progress to tumorigenesis.

FUTURE PERSPECTIVES

It is hypothesized that inflammatory mediators are ideal targets (biomarkers) for early detection, prevention, and therapy for many cancers and autoimmune diseases. Unique opportunities exist for designing effective strategies for diagnosis, intervention, and therapy at the various phases of inflammatory responses (Fig. 2). The design and development of a formula for a cancer risk index (CRI) based on using a combination of inflammatory mediators (eg, developing cytokine chips) and targeting mediators at various phases of inflammation may prove to be valuable approaches in

developing suitable diagnostic devices in clinical settings for inflammatory diseases and/or cancer^{26,27} (Khatami, personal communication).

A number of antiinflammatory drugs, including mast-cell stabilizers, inhibitors of prostaglandin or leukotriene synthesis (eg, COX or LO inhibitors), nitric oxide (NO) inhibitors, heparin inhibitors, therapeutic approaches for shifting the immune response from IgE to IgG, and a variety of antioxidants are the subject of extensive studies for detection and prevention of allergies and cancer^{19,30,39,40} (Khatami, personal communication). Several drugs that were originally used for the treatment of inflammatory diseases, such as asthma and conjunctivitis, and autoimmune diseases, such as rheumatoid arthritis, are currently being evaluated as potential chemopreventive agents for a number of cancers^{19,39} (Khatami, personal communication).

Following this same line of reasoning, design and implementation of cohort studies to follow a group of children or older adults with a wide range of allergic or autoimmune diseases may prove to be economically feasible approaches to detect cancer or even precancerous changes.

In conclusion, defining and understanding the dynamic profiles of inflammatory responses during developmental stages of inflammation-induced tumorigenesis should lead to better designs and strategies for the detection, chemoprevention, and therapy for many cancers.

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