



Review

Review: Diagnostic and therapeutic applications of rat basophilic leukemia cells

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ARTICLE INFO

Article history:

Received 28 March 2012

Received in revised form 30 May 2012

Accepted 31 May 2012

Keywords:

Rat basophilic leukemia (RBL) cell line
IgE high affinity receptor (FcεRI)

ABSTRACT

The research into understanding of the immunological processes is often difficult due to several factors complicating the isolation and culturing of primary degranulating cells like mast cells and basophils. The establishment of rat basophilic leukemia (RBL) cell line as an efficient and reliable experimental research tool was considered a major advance toward the understanding of the wild-type mast cell population's biology. The development of sub-clone RBL-IV (HR+) led to the isolation of histamine-secreting RBL-2H3 cell line. Since then, RBL-2H3 cells have been extensively used for studying the IgE high affinity receptor (FcεRI) interactions with their ligand, the IgE antibody. This cell line has been employed for generating human and more recently canine and equine FcεRIα-transfected RBL cell lines facilitating an assessment of the residues involved in the complementary interaction between the IgE molecules from these species and their cognate high affinity receptor. A proteomics-based approach to the definition of IgE-receptor-mediated signaling pathways was also carried out using this cell line. Furthermore, RBL-2H3 cells have the potential of being used to assess the potential allergenicity of antigens to humans and other animals like dogs and horses which are known to suffer from similar allergic manifestations.

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1. Introduction

Type I hypersensitivity is a generally accepted term employed to describe immunoglobulin (Ig) E-mediated allergic disorders. The first historical reference to an allergic response dates back to 2640 BC and depicts, on a pylon in Luxor, Egypt, the death of a Pharaoh from an anaphylactic shock following a bee sting (<http://www.slideshare.net/inemet/anaphylactic-reactions>). Current epidemiological studies show that there has been a dramatic increase in the occurrence of atopic diseases during the last quarter of the 20th century. The diverse ailments associated with allergy include atopic dermatitis, urticaria, allergic rhinitis, asthma and the potentially fatal condition of anaphylaxis (Corry and Kheradmand, 1999). Allergic diseases are now on the rise globally (Pawankar et al., 2008) and continue to constitute a serious health problem in industrialized countries. Worldwide 10–30% of the population has been reported to suffer from allergic manifestations of which the increase in childhood asthma gives rise to serious concern (Sole et al., 2001).

Immunoglobulin E and mast cells/basophils are key players in the allergic response (Williams and Galli, 2000). The symptoms

of immediate hypersensitivity are caused by the exocytic release of preformed and newly synthesized mediators that are secreted by mast cells and basophils in response to a variety of stimuli (Blank and Rivera, 2004). The related cellular process characterized more comprehensively involves the sensitization of mast cells and basophils by IgE bound to high-affinity IgE receptors. Subsequent receptor activation by cognate antigen–allergen leads to the regulated secretion of the pharmacologically active mediators responsible for the symptoms of class I hypersensitivity responses (Nadler et al., 2000). There is compelling evidence for the role of mast cells in allergy and asthma (Bradding et al., 2006) along with their function in different disease and immunological processes such as tissue remodeling, wound healing, pathological fibrosis, arthritis, angiogenesis and immune responses to neoplasia (Benoist and Mathis, 2002). Antigen-mediated aggregation of IgE bound to its high-affinity receptor on mast cells and basophils initiates a downstream signaling cascade leading to the exocytosis of preformed as well as de novo synthesized mediators which are responsible for the immediate and delayed symptoms associated with type I hypersensitivity responses (Siraganian, 2003).

The research into understanding of the immunological processes is often difficult due to several factors complicating the isolation and primary culturing of degranulating cells like mast cells and basophils (Passante et al., 2009). Cell culture is a useful technique available for carrying out research on eukaryotes, especially animal cells, under controlled and reproducible conditions in

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laboratory incubators. Cells obtained from tissues will continue to grow if provided with the appropriate environmental conditions such as optimal temperature and nutrients. The original aim of cell culture experiments was to study the biology of cells of a specific type under a simulated natural environment in order to improve the understanding of their normal physiology and biochemistry. Nowadays, the scope has been broadened by including the study of potential applications of their role in pharmacology and biotechnology. Primary peritoneal mast cells are comparatively easy to obtain by peritoneal lavage of small animals like rodents but tend to lose their response to antigenic stimuli during the purification process (Coutts et al., 1980). They are also difficult to maintain in primary culture for extended periods of time (Horigome et al., 1994). Initially, researchers probing mast cell biology relied on the use of connective tissue mast cells (CTMC) which could be obtained with ease from the peritoneal cavity in contrast to the mucosal mast cells (MMC) which were disfavored due to the problems encountered in their isolation (Befus et al., 1982).

Keeping in mind the above mentioned impediments, the arrival on the scene of the rat basophilic leukemia (RBL) cell line was considered a major advance toward the understanding of the mast cell prototype. RBL cells were developed in 1973 by inducing leukemia in rats fed with the chemical carcinogen, β -chloroethylamine (Eccleston et al., 1973). This newly generated cell line, labeled as RBL-1, although it expressed the high affinity receptor for immunoglobulin E (Fc ϵ RI) and displayed mast cell characteristics, was not secretory in nature and thus not of much use in degranulation studies (Siraganian et al., 1975). This challenge provided the stimulus for the development of various sub-clones. It was not until 1976 that a sub-clone RBL-IV (HR+) led to the isolation of histamine secreting RBL-2H3 clone (Barsumian et al., 1981). Since then, the RBL-2H3 cell derived lines have been used extensively for the study of IgE high affinity receptor (Fc ϵ RI) interactions with its ligand the IgE antibody. Single RBL cells have been shown to express approximately 500,000 Fc ϵ RI receptors on their surface (Ortega et al., 1988; Bingham et al., 1994). It was because of the RBL-2H3 cell line that the high affinity interaction of IgE with its receptor Fc ϵ RI was elucidated (Kulczycki et al., 1974). Similarly, the multimeric nature of Fc ϵ RI receptor complex was also demonstrated in this cell line (Holowka et al., 1980; Goetze et al., 1981). In view of their similar granular content to mast cells, RBL-2H3 cells are commonly employed as a prototypic and convenient model to study IgE mediated degranulation of mast cells, although in some aspects they have similarities to basophils rather than other histamine-releasing cell types (Passante et al., 2009). Receptor cross-linking activates signaling pathways that culminate in degranulation of multiple mediators (histamine, 5-HT, β -hexosaminidase) in an almost identical manner and constitution to the degranulation mechanism observed in primary mast cells and basophils (Barsumian et al., 1981; Funaba et al., 2003). This cell line has also been employed in assessment studies for mast cell stabilizers (Ikawati et al., 2001). The RBL model is expected to continue to serve in future as the model system for studying the biological characteristics of mast cells together with an additional potential role in clinical research (Blank and Varin-Blank, 2004).

2. Applications of rat basophilic leukemia cells in diagnosis and therapy

2.1. Generation of human, canine and equine Fc ϵ RI α -transfected RBL cell lines

The RBL-2H3 cell line is an established model for studying the secretory mechanism of histamine release, due to the relatively simple methods required to maintain them in culture as well as

the speed of growth into a confluent population (Bingham et al., 1994). These cells express all the subunits of IgE high-affinity receptor, but human IgE can only engage primate IgE receptors. RBL cells were transfected with the cDNA sequence encoding the ligand binding domain of the human IgE receptor, Fc ϵ RI α . The attempt to express the aforementioned chimeric receptors was also based on the significant molecular homology of the transmembrane and intracellular regions of the receptor alpha chain. This approach led, initially, to the generation of a stable cell line expressing a functional receptor complex bearing the human alpha chain and capable of binding human IgE and eliciting a mediator release in response to sensitization of the transfected cells with human IgE and subsequent cross linking with cognate antigen (Gilfillan et al., 1992; Wilson et al., 1993).

This success led to the generation of analogous cell lines, expressing the dog and horse Fc ϵ RI α chains which, like human Fc ϵ RI α , interact with the endogenous wild type rat beta and gamma chains to create a functional receptor complex which transmits an IgE-mediated stimulus to mediator secretion. The corresponding cloning protocols included codon optimization for the rat system before the new RBL cells were established, based on the experience gained from the initial success with the human Fc ϵ RI α transfection studies. The cells were shown to express the receptor on their surface using FACS, while release assays were used in order to assess proper receptor function in triggering degranulation and release of immunological mediators following antigen-specific stimulus. Thus, we developed stable RBL cell lines that express the human/dog/horse Fc ϵ RI α chain while providing experimental evidence which suggest that the IgEs from different species employ a common mechanism to activate target cells via their cognate receptor. Human IgE binds only human/primate α -chain (Gilfillan et al., 1992; Wilson et al., 1993) while rodent IgE binds human, dog, horse and rodent α -chain (Ye, 2011). Dog IgE binds human, dog and horse α -chain (Ye, 2011) and horse IgE binds horse and human α -chain (Sabban, 2011; Ye, 2011).

3. The high affinity receptor for IgE as a target for therapeutic intervention strategies

The precise events involved in stimulus-specific secretion triggered by coupling of sensitized high affinity receptors for IgE remain largely unresolved. Most inhibitors of mast cell degranulation are non-specific immunomodulatory drugs and their actions are likely to affect a number of physiological functions. It is therefore not surprising that their clinical application is associated with undesirable side effects. In order to increase safety, the identification of specific targets for therapeutic intervention is essential. We propose that a characterization of the contact sites between the transmembrane domains of the receptor subunits by mutational analysis of key amino acids may identify specific aggregation sites crucial for downstream signaling from the receptor complex. This information can be employed as the basis for the design of intermolecular wedges capable of inhibiting receptor aggregation by blocking contact sites between the receptor subunits. The high-affinity receptor complex for IgE plays a pivotal role in allergic responses since cross-linking of the high-affinity IgE receptor (Fc ϵ RI) on target cells initiates a signaling cascade facilitating release of inflammatory mediators causing allergic responses. The transmembrane regions of the ligand binding domains of the high-affinity IgE and low-affinity IgG receptors share an invariant motif (LFAVDVTGL) containing a polar aspartate within a predominantly non-polar setting (Ravetch and Kinetic, 1991). The functional importance of this aspartate residue (D194) in Fc ϵ RI-mediated receptor signaling was assessed by site-directed mutagenesis (Rashid et al., 2010). Rat basophilic leukemia cells (RBL-2H3) transfected with the

human IgE binding subunit (FcεRI) incorporating polar substitutions like asparagines (D194N) or threonine (D194T) resulted in the formation of a functional rat/human chimeric receptor complex. When activated via human IgE and cognate antigen, cells transfected with the former receptor variant resulted in mediator release, intracellular calcium mobilization and tyrosine phosphorylation of γ -chain and Syk kinase while a non-polar substitution (D194L) gave rise to cell surface expression of the mutated receptor subunit but failed to initiate downstream signaling. No cell surface expression of huFcεRI α gene constructs was observed when D194 was replaced with the non-polar Ile (D194I) residue of similar size, the larger positively charged Arg (D194R) or lysine (D194K) residues, nor the negatively charged glutamate (D194E) and smaller polar Ser (D194S) non-polar Ala (D194A) and Val (D194V). These observations highlight the importance of the size and charge of amino acid residue at position 194 in affecting IgE receptor subunit interactions, cell surface localization, and initiation of downstream signaling events (Rashid et al., 2010).

4. A proteomics based approach to the definition of IgE-receptor-mediated signaling pathways

In response to the activation of the IgE receptor complex, mast cells and basophils rapidly (~300 s) secrete powerful pharmacologically active mediators. Our recent study on FcεRI-mediated signaling in RBL-2H3 cell line demonstrated a novel central role for Stathmin 1 as an attenuative protein at regulation of IgE-mediated compared to IgE+ Ag mediated signaling in this cell line. This suggests that activation of single receptor by various stimuli could be regulated by alternatively modified molecules (e.g. Stathmin) to result in various responses (Sadroddiny et al., 2011). A time course study of the RBL cell's secretome in response to an IgE-mediated, antigenic stimulus has revealed a considerable diversity in the pattern of secretory proteins (Sadroddiny et al., 2012a). A further comprehensive kinetic analysis to identify changes on 2-D maps is likely to identify further unidentified proteins involved in downstream signaling and the induction of changes in the transcriptome of activated cells (Sadroddiny et al., 2012b). We have previously shown changes in the expression and secretion of cytokines/chemokines in activated RBLs using RT-PCR and immunological methods (Dudler et al., 1995; Machado et al., 1996; Smyth et al., 2000) but a more detailed analysis employing modern proteomic/transcriptomic techniques is required to generate a more global image of alterations in protein expression and secretion. The identification of such proteins should assist the elucidation of their function in mast cell/basophil physiology and expedite a focused search for homologous proteins in primary mast cells.

5. Applications: identification of the causative agent of an individual's allergic response

Currently, the most common means of identification of the allergen responsible for causing the symptoms of allergy in a patient is assessed either by skin prick testing, which carries the danger of causing a strong, adverse immune reaction or giving a 'boost' to an already sensitized individual, or by measuring allergen specific IgE from the patient's serum. Attempts to develop a human basophil degranulation test by Shelley (1974) proved unsuccessful because the technique was cumbersome, complicated and of low reproducibility. An automated spectrofluorimetric assay to determine histamine release in the presence of antigen also proved temperamental (Siraganian, 1977).

Although blood levels of IgE of defined antigenic specificity can be identified by a variety of immunological assays, the relevance of

this approach has been questioned arguing that most of a patient's IgE is bound to cellular receptors due to the high affinity between IgE and FcεRI and serum levels considered an unlikely indicator of a patient's allergic status. However, any IgE antibody in the circulation that is directed against an allergenic epitope forms part of a pool which can sensitize cells expressing the cognate receptors *in vivo* or *in vitro*. We have demonstrated the possibility of employing human FcεRI α -transfected RBLs to assess human IgE-mediated, allergen-induced histamine/ β -hexosaminidase release in response to known allergens (Wilson et al., 1993). The assay system is an attractive one and the advantages of such approach are several: good reproducibility, simplicity, no need to perform the assay within 48 h, no requirement to dissociate bound IgE from cells when assessing the binding of different ligands to FcεRI and no risk to the patient. The system, therefore, holds the promise to identify a patient's allergen susceptibility following the isolation of their IgEs to be used for sensitization of human α -chain transfected cells and subsequent challenge with a panel of allergens, giving rise to high mediator release (>30% of cellular mediators) reflected by a bell shaped dose response curve, characteristic of receptor mediated cell activation (Wilson et al., 1993).

One of the unexpected observations using this method, however, was the finding that many of the allergens under investigation were also able to induce low level mediator release (<10%) even in the absence of sensitization with cognate allergen. This difference cannot be identified in the skin prick test method, where mediator release is monitored in response to challenge with a panel of allergens. Any mediator release observed is attributed to cell sensitization followed by with allergen specific IgE. This surprising observation led us to investigate the possibility that allergens might interact with cells of the immune system, like mast cells and basophils, to generate the initial signals for subsequent adaptive immune responses.

6. Assessment of potential allergenicity

Atopic disease is characterized by an increase in the levels of synthesis of antibodies of the IgE isotype in response to what is generally termed 'seemingly innocuous antigens'. A combination of genetic and environmental factors has been invoked to explain the increased incidence of the disorder in the last five decades. Considering Japan, where the gene pool of the population has remained particularly stable, the dramatic increase in allergic manifestations suggests that environmental factors are the major cause. In mammals, a number diverse antigens act as allergens. These include insect venoms, plant pollens, fungal spores, latex associated proteins, house dust mite and cockroach emanations (reviewed in Dudler et al., 1995; Machado et al., 1996). In addition, there is evidence that pollutants in air such as diesel exhaust particles, increased concentrations of ozone and oxygen radicals produced by engine emissions or cigarette smoke act as adjuvants to create an environment capable of enhancing IgE synthesis (Smyth et al., 2000).

It is not unusual for exposure to substances at the workplace leading to the development of occupational allergies. These allergies have a considerable socio-economic impact since they can be severely debilitating, reducing work performance and quality of life of afflicted individuals. Interestingly, the rise in childhood atopy mirrors the widespread introduction of diesel engine powered cars and is frequently associated with the inability to attend school and reduced academic performance. Collectively allergic manifestations place an ever increasing burden on public health care.

However, as a result of the diverse nature of substances linked to the development of allergic disease, no unifying feature regarding the nature of antigens and pollutants has emerged from

the numerous studies investigating the molecular structure and diverse composition of many diverse allergens. Consequently, there is no accepted means of predicting or assaying for the potential allergenicity of industrial and environmental agents although such information could be applied in a proactive manner to prevent exposure before allergic sensitization occurs.

Knowledge of the cellular and molecular mechanisms involved in the pathogenesis of allergy is currently largely inadequate to give a complete description of events leading to the establishment of an allergic response. However, a regulatory role for cytokines has been clearly demonstrated with interleukin-4, 10 and 13 having a central role in the induction and maintenance of a cellular environment favoring IgE antibody responses, while IL-12 and interferon- γ play a role in the down-regulation of IgE synthesis. Underlying these changes in cytokine expression, the signals promoting the induction of pro-inflammatory cytokine synthesis and secretion as well as their place in the matrix of factors that determine allergenicity have still to be resolved.

Understanding the molecular basis of potential allergenicity should assist the implementation of test screen linked to avoidance strategies. IL-4 plays a pivotal role in the induction of class switching to the IgE isotype in B-cells, but the source of IL-4 prior to the establishment of T-cell responses is still uncertain. It is known that IL-4 is secreted from human basophils and mast cell lines in culture in response to an IgE-mediated antigenic stimulus.

Using the rat basophilic leukemia cell line as a model system to study mast cell function in culture, we found that these cells respond to the presence of common allergens and pollutants found in e.g. cigarette smoke or car emissions by synthesizing and secreting pro-allergenic cytokines including IL-4, IL-10, IL-13 even in the absence of sensitization with IgE (Dudler et al., 1995; Machado et al., 1996; Smyth et al., 2000).

The observation that many common allergens and environmental pollutants can trigger pro-inflammatory cytokine synthesis and secretion suggests that a bioassay could be used to predict the allergenicity of defined and complex mixtures of environmental agents. Preliminary observations show that cell preparations of human mast cells and basophils produce pro-inflammatory cytokines in response to provocation by the same allergens as used to challenge RBL cells (Machado et al., 1996). Our results therefore indicate that rodent and human mast cells exhibit similar responses to allergens and pollutants, which supports the notion that similar mechanisms operate in rodents and man with regard to the potential initiation of allergic responses.

The demonstration that allergens and pollutants induce cells of mast cell lineage to release pro-inflammatory cytokines into the extracellular fluid prior to sensitization with IgE has important implications. It is well established that in combination with IL-4/13 mast cells and basophils are able to furnish, via CD40L, the minimal signals necessary to induce class-switching to IgE in B-cells. Furthermore, IL-4 is known to induce the differentiation of uncommitted T helper cells into Th2 cells. In addition, the demonstration of cytokine release in response to e.g. cigarette smoke or diesel exhaust particles is highly significant since it explains reports that these pollutants can produce an environment capable of enhancing ongoing IgE synthesis.

Furthermore, we have recently demonstrated the presence of five receptors on RBL cells including the Fc ϵ RI- γ , PARs, glutamate [NMDA] receptor, low-density lipoprotein receptor (LDL-R), interleukin-1 receptor-like 1 which play an important role, together and alongside Fc ϵ RI, in the molecular mechanisms of the allergic response (Sadroddiny et al., 2012a). PAR-2 is commonly known as target receptor for mast cell tryptase on neighboring cells (Akers et al., 2000; Stenton et al., 2002). However, presence of PAR-2 on MC/B may further propose a self-regulatory mechanism for autogenous tryptase. This notion is further supported by studies

that show trypsin, chymotrypsin, thrombin and pro-allergenic proteases of e.g. house dust mite origin directly induce mast cell- and RBL cell secretion in the absence of IgE-mediated receptor activation (Corvera et al., 1997; Dugina et al., 2003; Machado et al., 1996). The expression of PAR-2 on mast cells may further explain the contribution of mast cells in inflammatory responses, tissue remodeling, and injury, attributable to their activation via PARs (Reed and Kita, 2004). This study also identified a group of cytokines, chemokines and growth factors such as platelet factor 4 (PF4), C-C motif chemokine 2 or monocyte chemoattractant protein 1 (MCP-1), macrophage colony-stimulating factor 1 (CSF-1) and transforming growth factor beta-1 (TGF- β 1). The biological roles of these proteins could be best explained by interaction of MCs/Bs with other cells of the immune system. For example, small inducible cytokine A2 or MCP-1 is able to recruit monocytes and memory T cells and dendritic cells to the site of tissue injury and infection (Houenou et al., 1996). Chen et al. (1998) showed a significant increase (21-fold) of mRNA tag sequence of MCP-1 after IgE-mediated activation of RBL-2H3 cells. The binding of IgE to Fc ϵ RI on dermal mast cells, even in the absence of antigen, has been shown to enhance mRNA for MCP-1 (Bischoff et al., 1999). Identification of MCP-1 in activated mast cells and basophils strengthens the claim for a role of these cells in regulation of cellular immunity in response to foreign stimuli.

Collectively, these observations indicate that we have obtained evidence for a pivotal role of cells of mast cell lineage and their mediators in the steering of subsequent adaptive immune responses and our work suggests that these cells may be employed to predict potential allergenicity by means of a biological assay system since any substance which induces cells to synthesize and secrete IL-4/13 must be considered potentially allergenic. To further substantiate the use of the RBL cell line as a predictor of potential allergenicity, the following parameters of cell activation can be assessed:

- Mediator (5-HT or histamine) release.
- Cytokine synthesis assessed by RT-PCR of RNA extracted from cells for an array of pro-inflammatory cytokines.
- Cytokine secretion by ELISA.
- Protein phosphorylation.
- Changes in intracellular Ca metabolism.

The application of proteomic techniques may assist in answering the questions whether mediator synthesis and secretion is due to the induction of identical or diverse pathways of signal transduction and lead to the identification of the best indicator/readout of cell activation and by inference, potential allergenicity.

Resolving these issues can be expected to provide the scientific foundation for the establishment of a biological assay system for the routine assessment of the potential allergenicity and/or IgE adjuvant activity of allergens/pollutants and help to assess the risk of allergenicity associated with e.g. genetically engineered foods. It is interesting to point out, by analogy, that a biological assay system for the screening of potential carcinogenicity, such as the Ames test, is already employed. The development of a bioassay, based on RBL-2H3 cells, for the correlation of potential allergenicity may be applied in a pro-active manner and prevent inadvertent exposure to compounds which may lead to severely debilitating allergic manifestations which reduce work-performance, quality of life and place an ever increasing burden on public health care.

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