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Research paper

Transient transfection of human peripheral blood basophils

Natalia Vilariño, Donald MacGlashan Jr.*

Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, United States

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Abstract

The human basophil has resisted previous attempts at transient transfection. Basophils were transfected by nucleoporation and to test whether there was sufficient expression to modify cell function, the cells were transfected with a syk kinase tandem SH2 construct linked to GFP. This approach was taken because in RBL cells and murine mast cells syk kinase is known to play a very early role in signal transduction and previous studies in RBL cells demonstrated that expression of the tandem SH2 domains of syk would inhibit signaling, presumably by competition with endogenous syk for binding to ITAMs. Results from basophil transfections with $SH2^{syk}$ were compared to an empty construct. Basophils were stimulated with anti-IgE antibody and analyzed for single cell changes in cytosolic calcium levels. Basophils expressing the empty GFP construct showed a cytosolic calcium response similar to non-expressing cells. In contrast, basophils expressing the GFP-tandem $SH2^{syk}$ construct, on average, showed an anti-IgE-induced calcium response that was completely ablated. The transfection frequency was 8% (median), with an average viable recovery of 12% (n=18). While the procedure is not benign and is not always successful, these studies indicate that with gating techniques, the human basophil, a non-dividing primary leukocyte, can be transiently transfected to express high enough levels of an inhibitory protein to alter an IgE-mediated response.

Keywords: Mast cells/basophils; Allergy; Signal transduction; Primary cells

1. Introduction

The basophil and mast cell are considered central to immediate hypersensitivity reactions due to their expression of high affinity receptor for IgE antibody (FcεRI) and their secretion of mediators like histamine following aggregation of FcεRI by antigen. There are several animal and cell line models that have provided a considerable background of knowledge on the signal transduction steps involved in IgE-mediated signaling (Li et al., 1992; Stephan et al., 1992; Pribluda et al., 1994; Hirasawa et al., 1995; Rivera and Brugge, 1995; Jabril-Cuenod et al., 1996; Zhang et al., 1996; Parravicini et al., 2002). One reason for progress occurring in these models is the ability to transfect the cells with plasmids that generate modified proteins involved in signal transduction. Often, with some

* Corresponding author. Tel.: +1 410 550 2145; fax: +1 410 550 2130.
E-mail address: dmacglas@jhmi.edu (D. MacGlashan).

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caveats, this is necessary to test causal relationships in signaling components. Mirroring these studies using human cell equivalents, the tissue mast cell or circulating basophil, has not been possible unless cell permeable pharmacologic agents are available. Although we have done a variety of signal transduction studies using human basophils as a model (MacGlashan and Guo, 1991; MacGlashan and Hundley, 1994; Kepley et al., 1998; Miura and MacGlashan, 1998; Miura et al., 1999; Miura and MacGlashan, 2000; Miura et al., 2001a,b; MacGlashan, 2003; Vilarino and MacGlashan, 2004), these studies have been limited by the inability to transfect the cells. There are no published successes of transfection and in our own hands, a variety of techniques (electroporation, detergents, anti-sense, viral vectors) have been tried over the last two decades but without success (unpublished data). More recently, we have explored, also without success, the use of TAT-tagged proteins (unpublished data). However, a modification of the common technique of electroporation, termed nucleoporation, has been found to result in the introduction of plasmids into the nuclei of non-dividing cells. This technology has been applied to the problem of human basophil transient transfection resulting in modest rates of transfection that are sufficient, with gated analysis, to measure alterations in IgE-mediated signaling.

As a test case, syk kinase was examined for its role in directing the cytosolic calcium response in basophils. Syk kinase has a well-documented role as one of the earliest steps in IgE-mediated signaling in murine and rat mast cells (Stephan et al., 1992; Oliver et al., 1994; Rivera and Brugge, 1995; Chen et al., 1996; Costello et al., 1996; Jabril-Cuenod et al., 1996; Zhang et al., 1996; Zhang et al., 2001). Less direct studies also indicate a role for syk kinase in IgE-mediated signaling in human mast cells and basophils (Suzuki et al., 1997; Kepley et al., 1998; Kepley et al., 1999; Lavens-Phillips and MacGlashan, 2000; Miura and MacGlashan, 2000; Miura et al., 2001a,b). To test both the ability to modify signaling and to further test the role of syk kinase in the IgE-mediated reaction, the tandem SH2 domains of syk were used as a competitive inhibitor of the endogenous syk kinase (Taylor et al., 1995). This technique was previously demonstrated to alter the IgE-mediated response of RBL cells (Taylor et al., 1995).

2. Materials and methods

2.1. Materials

The following were purchased: PIPES, bovine serum albumin (BSA), EGTA, EDTA, D-glucose, FMLP (Sigma, St. Louis, MO); crystallized human serum albumin (HSA) (Miles Laboratories, Elkhart, IN); fetal calf serum (FCS) and RPMI 1640 containing 25 mM HEPES and L-glutamine (BioWhittaker, Walkersville, MD); Percoll, (Pharmacia, Piscataway, NJ); Tris (hydroxymethyl)-aminomethane, Tween-20 (Bio-Rad, Hercules, CA).

2.2. Buffers

PIPES-albumin-glucose (PAG) buffer consisted of 25 mM PIPES, 110 mM NaCl, 5 mM KCl, 0.1% glucose, and 0.003% HSA. PAGCM was PAG supplemented with 1 mM $CaCl_2$ and 1 mM $MgCl_2$. PAG-EDTA consisted of PAG supplemented with 4 mM EDTA. Countercurrent elutriation was conducted in PAG containing 0.25% BSA in place of 0.003% HSA.

2.3. Basophil purification

For most of these experiments, residual cells of normal donors undergoing leukapheresis were enriched in basophils using a combination of Percoll density gradients and countercurrent-flow elutriation, as previously described (MacGlashan et al., 1994). We used a cocktail of antibodies for negative selection from Stem Cell Technologies (Vancouver, BC) (basophil purification kit) and columns from Miltenyi (Aubum, CA). The purity of basophils was determined by alcian blue staining (Gilbert and Ornstein, 1975) and basophils purified from leukapheresis packs generally exceeded 99% purity. In some experiments, basophils were enriched to near homogeneity from blood obtained by standard venipuncture as described previously (Lavens-Phillips and MacGlashan, 2000). Briefly, the basophils were first enriched by separation on a two-step Percoll gradient followed by negative selection using the same reagents and columns described above for purification of basophils from leukocyte packs.