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IMMUNOCHEMICAL STUDIES ON KENTUCKY BLUE GRASS POLLEN ALLERGENS

by

Subhas Chakrabarty B.S., M.S.

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Submitted to the Faculty of Graduate Studies in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DOCTOR OF PHILOSOPHY

TO MY WIFE LILA

HER SUPPORT AND UNDERSTANDING HAVE MADE THIS WORK POSSIBLE

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SCOPE OF THE INVESTIGATION

The principal objectives of this investigation were to (i) isolate haptenic components from Kentucky Blue Grass (KBG) pollen which are able to block the allergic reactions triggered by multi-valent allergens of KBG pollen; (ii) isolate an allergenic component in pure form from KBG pollen and to characterize this component; (iii) examine the allergenic and antigenic relationships of these components employing murine IgE antibodies, human IgE antibodies and rabbit precipitating antibodies.

This thesis is divided into four chapters. The first chapter is introductory in nature and contains a literature survey and general information.

The second chapter deals with methods of isolation of haptenic components from KBG pollen and the biological properties of these components.

The third chapter summarizes results of studies on the isolation and characterization of a purified allergen from KBG pollen.

The last chapter examines the allergenic and antigenic relationships of two purified allergens isolated from KBG pollen and the antigenic relationship of the haptenic components to these two purified allergens.

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ABSTRACT

Components with haptenic properties were isolated from the non-dialyzable fraction, i.e. the retentate (R) and the dialyzable fractions of the aqueous extract of Kentucky Blue Grass pollen (KBG aq. ext.) by preparative isoelectrofocusing (Prep-ISO-EF) on Sephadex G-100 gel. The haptenic components could not elicit PCA reactions in rats passively sensitized with murine reaginic antisera to R but they could inhibit completely and specifically the PCA reactions normally elicitable with R. Some haptenic fractions which contained only a few components, detectable by analytical isoelectrofocusing (Anal-ISO-EF), could inhibit specifically and completely the PCA reactions normally elicitable with R which contained over 30 components. This clearly indicated that such haptenic fractions possessed all the allergenic specificities present in R. It was concluded that the specificity of murine IgE antibodies was directed to a determinant(s) which was common to either

allergenic or haptenic fractions. On the other hand, by employing a pool of human sera from individuals allergic to KBG pollen in the RAST procedure, it was apparent that most of the haptenic fractions lacked some of the specificities present on allergenic components of R that are recognized by the human IgE antibodies.

An allergenic glycoprotein (M.W. 11,000) designated as Allergen C was isolated from R by a combination of Prep-ISO-EF and gel filtration on Bio-Gel P-60. It possessed all the allergenic specificities of R recognized by the murine reaginic anti-R sera. On the other hand, Allergen C lacked some of the specificities recognized by the pool of human allergic sera. Allergen C contained all the naturally occurring amino acids with the exception of cysteine. The allergenic activity was found to be stable on exposure to extremes of pH and guanidine HCl treatment; since protease treatment completely destroyed its allergenic activity, it is suggested that allergenicity is associated with the protein moiety of this molecule. Enzymatic digestion of Allergen C revealed that the allergenic determinants recognized by the murine IgE antibodies were different from the antigenic determinants recognized by a rabbit precipitating antiserum to Allergen C.

Allergen C and another allergen, KBG-1 also isolated from KBG aq. ext. were found to be allergenically identical

in terms of their specificities evaluated with the murine reaginic sera; whereas they were only partially identical when evaluated with the human allergic sera. However, Allergens C and KBG-1 were antigenically distinct with respect to rabbit precipitating antisera produced separately to each allergen. Allergen C did not share any common antigenic specificities with the haptenic components; however, Allergen KBG-1 were found to share some common antigenic specificities with the haptenic components.

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ABBREVIATIONS

Anal-ISO-EF	Analytical isoelectrofocusing
BSA	Bovine serum albumin
CIE	Crossed immunoelectrophoresis
cpm	Counts per minute
CRIE	Crossed radio-immunoelectrophoresis
D_{24}	24 hour dialysate of KBG aq. ext.
D_{48}	48 hour dialysate of KBG aq. ext.
DNP	Dinitrophenol
hrs.	Hours
i.p.	Intraperitoneal
i.v.	Intravenous
KBG aq. ext.	Aqueous extract of Kentucky Blue Grass pollen
MW	Molecular weight
mins.	Minutes
OA	Ovalbumin
PCA	Passive cutaneous anaphylaxis
P-K transfer test	Prausnitz-Küstner transfer test
Prep-ISO-EF	Preparative isoelectrofocusing
R	Retentate or non-dialyzable constituents of KBG aq. ext.
RAST	Radioallergosorbent Test
RIA	Radioimmunoassay
RPM	Revolutions per minute
secs.	Seconds
TCA	Trichloroacetic acid
V	Volts

CHAPTER 1

INTRODUCTION

IgE MEDIATED HYPERSENSITIVITY

Hypersensitivity can be defined as an altered state of immunity induced by an antigen in which anaphylactic reactions and symptoms of atopic sensitivity can be subsequently elicited by that antigen, or by structurally similar substances. At the end of the 19th century following the discovery of antitoxins and antimicrobial antibodies, the nature of the immune response was considered to be purely protective. However, at the beginning of this century it was realized that immune responses also possessed harmful potentialities. Portier and Richet (1902) proposed that certain immunological mechanisms similar to those involved in protection from microbial infection could also result in harmful reactions and effects which could be fatal. At the beginning of the 20th century, von Pirquet (1906) coined the term "allergy" (Greek for "altered action") to denote any harmful altered response to a substance induced by previous exposure to it. Through usage, "allergy" and "hypersensitivity" have become synonymous: both referred to the harmful altered response to a substance induced by previous exposure.

Hypersensitivity states can be classified according to the onset of the reaction following exposure of sensitized individuals to the offending antigen. Sensitization refers to the altered state of immunity induced by exposure to the offending antigen. The two main classes of hypersensitivity are: (i) of the immediate and (ii) of the delayed type. Immediate type hypersensitivity can be passively transferred with serum from a sensitized individual to a non-sensitive recipient and it is due to the presence of humoral antibodies in the serum. Anaphylaxis and atopic sensitivity are two examples of immediate type hypersensitivity. By contrast, humoral antibodies are not involved in delayed type hypersensitivity and its passive transfer is usually accomplished with white cells from a sensitized individual or with soluble extracts of these cells. Examples of delayed hypersensitivity are: tuberculin sensitivity, contact allergy, and phenomena related to transplantation immunity. The manifestation of different forms of immediate type hypersensitivities are described below.

Anaphylaxis

The term "anaphylaxis" was coined by Portier and Richet (1902) (Greek, ana = against; phylaxis = protection) to denote an increase in susceptibility to a toxic substance rather than the expected increase in resistance. They observed that dogs given a second injection of sea

anemone extract, several weeks after the first, became acutely ill, went into shock and died within a few minutes. Other investigators (Koch, 1890; Flexner; 1894) in the late 19th century had also observed similar phenomena upon reinjection of antigens into previously sensitized animals. When an animal is sensitized with an antigen, an interval of time is required before anaphylactic shock can be elicited on reinjection of the antigen into the animal. During this interval antibodies are formed which become fixed to tissues (target organ) rendering that animal prone to anaphylaxis. When the antigen is injected into the sensitized animal, the combination of antigen with tissue-fixed antibodies leads to the release of pharmacologically active agents [primarily histamine, slow reacting substance of anaphylaxis (SRS-A), serotonin (5-hydroxytryptamine), eosinophilotactic factor of anaphylaxis (ECF-A), kinins, and prostaglandins] from target cells. These mediators diffuse through the extracellular fluid surrounding the target cell until contact is made with certain effector structures - smooth muscles and blood vessel walls. Contraction of the former and enhanced permeability of the latter give rise in turn to clinical symptoms characteristic of immediate hypersensitivity (wheal and flare skin reactions, itching, sneezing etc) or anaphylactic reactions.

Atopic Sensitivity

The term atopy (meaning strangeness) was introduced

by Coca and Cooke (1923) to describe a type of hypersensitivity occurring mostly in man. Asthma, hayfever, urticaria, angiodema are some of the chief atopic conditions. The allergens responsible for this type of hypersensitivity are found in pollens, feathers, animal danders, house dusts, and in food such as milk and eggs. Simple chemicals and drugs such as penicillin which can react with the host's protein can also cause atopic sensitivity.

Prausnitz and Küstner (1921) first recognized that the condition of immediate hypersensitivity was brought about by the production of a serum factor which could be detected in the serum of allergic individuals. They demonstrated that the allergic reactivity of an atopic patient could be passively transferred with the patient's serum to the skin of a normal individual, i.e. the injection of the allergen into the sensitized skin sites resulted in an inflammatory reaction similar to that obtained on injection of the allergen into the skin of the allergic patient. This passive transfer test, referred to also as the Prausnitz-Küstner (P-K) test was the first test for the demonstration of skin-sensitizing antibodies in the sera of allergic individuals. These skin-sensitizing antibodies were designated by Coca and Grove (1921) as reagin. Skin-sensitizing antibodies were found not to be able to cross the placenta and were retained by the choroid plexus (Bell and