INCREASED HYALURONIC ACID IS ASSOCIATED WITH DERMAL DELAYED-TYPE HYPERSENSITIVITY

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Abstract—Rabbits sensitized subcutaneously with heat-killed bacilli Calmette-Guerin (BCG) and challenged intradermally with heat-killed BCG or purified protein derivative (PPD) demonstrated classical dermal delayed-type hypersensitivity reactions which peaked two days postchallenge. Animals challenged with BCG developed dermal granulomas as measured by induration and gross observation. Challenge with either PPD or BCG resulted in increased levels of dermal hyaluronic acid (HA) by two days postchallenge. Dermal HA returned to normal levels by seven days postchallenge regardless of the challenge antigen. These results indicated that increased HA is associated with dermal delayed-type sensitivity, but increased HA is not associated with dermal granulomatous hypersensitivity. These results are in contrast to previously reported work which indicates that increased HA is associated with both pulmonary delayed hypersensitivity and pulmonary granulomatous hypersensitivity.

INTRODUCTION

Our laboratory previously described a macrophage-agglutinating factor (MAgF) obtained from lung lavages of bacillus Calmette-Guerin (BCG) -sensitized rabbits 2 days after intratracheal challenge with heat-killed BCG (1). These conditions cause a delayed-type hypersensitivity (DTH) response which subsequently develops into pulmonary granuloma formation. Chemical analyses and enzyme susceptibility studies identified the agglutinating component of MAgF as hyaluronic acid (HA) (2). Further studies indicated that HA increases to maximal levels in the lung lavages and lung parenchyma by 2 days after intratracheal challenge with BCG or soluble tuberculosis protein (TP) (3). No other glycosaminoglycans (GAGs) are detected in the lung lavage.

When BCG is the challenge antigen, HA remains elevated in the lung
parenchyma, but not in the lavageable fluids, for at least 21 days, while TP-challenged animals demonstrate normal HA levels in both lung lavage fluids and lung parenchyma by 5–7 days postchallenge (3). Hyaluronic acid is a minor GAG in the lung tissue, but no discernable pattern is observed with heparan sulfate, dermatan sulfate, and the chondroitin sulfates.

The increases in HA seen in lung lavage and lung parenchyma and the agglutination of macrophages suggest that HA might participate in the early events of cellular organization during granuloma formation. The purpose of this study was to determine if HA might play a similar role in dermal DTH and granuloma formation. HA was isolated from the dermal and subdermal tissue of sensitized rabbits undergoing reactions to purified protein derivative (PPD) and BCG. The results were compared to previous results in which the rabbit lung was used as a model. These studies demonstrated a correlation between the accumulation of HA and the expression of DTH, but no correlation was observed between the accumulation of HA and progression of the granulomatous lesions.

MATERIALS AND METHODS

Animals. Female outbred New Zealand white rabbits weighing 2–3 kg were used throughout this study. All animals were maintained in the Department of Comparative Medicine.

Antigens. Heat-killed BCG was obtained by culture of Mycobacterium bovis, strain bacillus Calmette-Guerin (BCG), in Proskauer-Beck medium (4) for 6 weeks, followed by washing with phosphate-buffered saline (PBS), then autoclaving. Killed BCG was lyophilized to dryness and stored at 4°C. Purified protein derivative (PPD) was obtained from Parke, Davis and Company (Detroit, Michigan).

Induction of Responses. Rabbits were sensitized subcutaneously at the base of each ear with 100 μg of heat-killed BCG suspended in Marcol 52 as previously described (1). The ear pinna was used as the site for skin reactions as described by Schroff et al. (5). Three weeks after sensitization, the rabbit ears were shaved, and one ear was injected with either 30 μg of heat-killed BCG in 0.1 ml PBS or 20 μg of PPD in 0.1 ml PBS. The other ear was injected with 0.1 ml PBS as a control. Nonsensitized animals were injected with PPD or BCG as controls. The course of the skin reactions was measured with Schnellmater micrometer calipers (H. C. Kropelin Gmb H. Schultern, FRG) as described by Schroff et al. (5). Net induration of the reaction site was measured as thickness of the ear after challenge minus thickness of the ear before challenge.

The animals were sacrificed at 2, 7, and 14 days when BCG was the challenge antigen and at 1, 2, and 7 days when PPD was the challenge antigen. Each group of experimental animals included at least six rabbits, and each group of control animals included at least three rabbits.

Extraction of HA. Using a 1.8-cm-diameter cork borer, a plug of ear tissue was removed from the site of the antigen or PBS challenge. GAGs were extracted according to reported procedures (3, 6, 7). The plugs of tissue were shaved and minced with a scalpel to approximately 1-mm cubes. The tissue was homogenized at 4°C in normal saline with a Sorval Omni-Mixer and placed in a boiling water bath for 5 min to denature proteins.