NEW INSIGHTS IN PATHOPHYSIOLOGY OF CONTACT HYPERSENSITIVITY:

FROM TRANSGENIC MICE TO HUMAN PATHOLOGY

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Introduction

The skin represents a unique immunologic organ. It poises not only to protect the host from environmental antigens and invading organisms but is also an important target for a variety of allergic and auto-immune responses such as contact hypersensitivity (CHS). CHS is a T cell-mediated inflammatory reaction occurring at the site of challenge with a contact allergen in sensitized individuals (1).

A CHS reaction consists of two distinct phases: i) sensitization phase i.e. priming of naive T cells after epicutanous contact with hapten and ii) effector phase i.e. activation of specific T cells after subsequent contacts with hapten. While the sensitization phase is generally asymptomatic, the elicitation phse characterized by redness and vesicles, followed by scaling and dry skin that clinically known as allergic contact dermatitis.

At the cellular level CHS involves three elements: contact allergens (haptens), antigen presenting cells (APC) and T cells (hapten-Specific).

Haptens

Contact allergens are low molecular weight chemicals named haptens, that are not by themselves immunogenic. For being immunogen, haptens need to bind epidermal proteins (carrier) and to form the hapten-carrier complex (2). They furthermore, need to be processed by APC as a haptenated peptide for presentation to T cells.

Most haptens bear lipophilic residues, which enable them to pass through the corneal barrier, and electrophilic residues, which account for covalent bounds to the nucleophilic residues of cutaneous proteins. Haptens often derive from unstable chemicals, named pro-haptens, which require an additional metabolization step *in vivo* in the epidermis to be converted into the electrophilic antigenic hapten endowed with T cell antigenic properties. This is the case of urushiol (poison ivy) and of photosensitizers, which must be activated by UVlight in order to bind to epidemal proteins (3). Metal salts do not bind covalently to cutaneous proteins but form complexes with these proteins through weak interactions. Some metal salts also undergo chemical conversions in the skin, as

hexavalent chromium salts, which are turned into trivalent chromium in the epidermis with a high capacity to bind cutaneous proteins (4). Thus, the metabolic status of the host for a given hapten is a key determinant in development of allergic contact dermatitis for at least some contact allergens (5).

It is important to note that most haptens could also exhibit a dose dependant toxicity that may induce irritant dermatitis that is non-immunological in nature.

T lymphocytes

Activatioin of the naive T lymphocytes by langerhans cells having loaded the hapten in the epidermis occurs in the regional draining lymph nodes whereas activation of hapten-specific T cells usually occurs in the skin. CD8+ and CD4+ cells are the two major hapten-specific T cells found in Ch. S. The CD4+ cells produce type 2 cytokine and are strictly MHC class II-restricted, while CD8+T cells produce type 1 cytokine and are strictly MHC class I-restricted.

Priming of naive T cells by haptenated langerhans cells is believed to be consecutive to recognition by the T cell receptor of a conformational complex formed by the hapten-modified self peptide within the groove of both major histocompatibility complex (MHC) class I and class II molecules. After being processed by langerhans cells, a hapten could activate both hapten-specific class I-restricted CD8+T cells and class II-restricted CD4+ T cells and class II-restricted CD4+T cells. The CD8+T cells are effector cells that without them without them a CHS response could not be seen. The CD4+ T cells exert a down regulatory effect; removing these cells result in an exaggerated CHS response (6). By contrast, DTH is a response elicited by CD4+ T cells with apparent down regulatory effects of CD8+ T cells.

Antigen presenting cells

Antigen presenting cells have a key role in both sensitization anhd elicitation phases of CHS. While the langerhans cells represent the main APC cells for the sensitization phase, their presence are not necessarily required for the eliciation phase of CHS. Moreover, the dendritic group of APC other than epidermal langerhans cells, especially dermal dendritic cells, could also participate in the sensitization phase of CHS (7). The basic role of langerhans cells in initiation of CHS reactions has been clearly shown by several experiments. A CHS response could not be seen witout presence of langerhans cells (8,9). Additionally, it was shown that sensitization to a particular hapten could be transferred to naive mice by injection of haptenized langerhans cells (10,11).

Haptens could bind to cell surface proteins which are internalized and processed into peptides. These haptenated peptides can be eventually recognized by MHC class II-restricted CD4+T cells or MHC class I-restricted CD8+T cells. Alternatively, direct binding of haptens, without processing, to a peptide in the groove of either MHC class I or class II molecules may also contribute to recognition by CD8+ or CD4+T cells, respectively. We have recently shown that dendritic cells can present haptenated peptides by their both MHC class I and class II molecules and activate antigen-specific CD8+ effector and CD4+ regulatory T cell subsets, concurrently and independently (6).

Langerhans cells capture the hapten in the epidermis and migrate to the draining lymph nodes (12,13). Surface expression of adhesion molecules allows langerhans cells to migrate through the basal membrane into the dermis and down to the draining lymph nodes (14-16). Langerhans cell activation and migration appear to be under influence of cytokines and soluble factors such as TNF-α and IL-1(17).

Langerhans cells undergo morphological and phenotypical changes during their migration (18). Langerhans cells appear very efficient for both antigen processing and antigen presentation. The balance between these two functional properties would, however, be altered during the migration. More precisely, langerhans cells are particularly efficient for antigen processing in the epidermis where they take up the hapten. When langerhans cells migrate from the epidermis to the draining lymph nodes, atigen processing is less efficient,

but on the contrary, antigen presentation capacities is strongly increased. This allows activation of the naive hapten-specific T lymphocytes in the paracortical area of lymph nodes (19,20). In physiological conditions, langerhans cells are the only cells in the epidermis which constitutively express major histocompatibility (MHC) class II molecules, and expression of these molecules is strongly modified specifically by haptens in the CHS reaction and not by irritants (21-24).

CD1a molecule, which is another specific marker of langerhans cells in the epidermis, disappears from the cell surface during migration to the draining lymph nodes. Finally, some intracellular markers of langerhans cells are also altered, as ATPase activity which strongly decreases after loading of the hapten by langerhans cells(25).

Cell and molecular events leading to CHS

Events leading to CHS is divided in two distinct phases (Figure):

1. The sensitization phase, also referred to as induction phase lasts 10 to 15 days in man. In the epidermis, lanerhans cells take up hapten, process it and migrate to the regional draining lymph nodes, where they present the Ag to naive T cells. During this process, langerhans cells convert from a 'resting' into an 'activated' functional state. This langerhans cells activation is due to either i) deirect effect of haptens on langerhans cells or ii) the inflammatory cytolkines produced by keratinocytes as a result of hapten application. Activation and migration of langerhans cells appears to depend on the capacity of haptens to induce langerhans cells to secrete IL-1β. Thus, haptens, by virtue of their capacity to induce a specific cytokine pattern, are the primary stimulus that activates langerhans cells and induce the sensitization process (26). The first stimulus is, however, in-sufficient to cause an inflammation and the sensitization step has no clinical consequence.

once memory hapten-specific T cells were generated in the lymph nodes, they migrate back to the skin. The cutaneous lymphocyte antigen (CLA) is supposed to represent a major skin-homing receptor for T cell migration to the skin (27,28). Selective trans-endothelial migration of memory/effector T cells makes possible after CLA (expresses on T cells) interaction with E-selectin (expresses on endothelial cell layers). The receptor-ligand pairs VLA - 4/VCAM-1 and LFA-1/ICAM-1 are also involved in this process.

2. The elicitation phase (also known as effector or challenge phase) a is T cell dependant mechanism that takes 72 hours to be developed. Two singals seem to be required for elicitation of a hapten-specific CHS:i) Ag-specific signal and 2) a pro-inflammatory signal (Ag non-specific) (26). Direct pro-inflammatory effect of hapten on epidermal cells including expression of adhesion molecules, MHC and cytokine production may initiate the cascade. These would result in a minimal cellular infiltration at the site of hapten application. In case that already sensitized T cells are present in the skin, hapten could further activate these primed T cells. Hapten-specific T cell activation is followed by cytokine production, especially IL-1, IL-2, IFN-γ and TNF-α and activation of other cell types, particularly keratinocytes and endothelial cells. This would further attract additional cells resulting in clinically manifest allergic contact dermatitis.

The relative contribution of the different epidrmal and dermal cell types in the activation of effector T cells is still a matter of debate. In contrast to the sensitization phase that langerhans cells play a major role in Ag presentation to T cells, langerhans cells are not necessarily required for the elicitation phase of CHS. Activated keratinocytes and macrohages present at the lesional site may also function as APC. Activated keratinoucytes express surface ICAM-1 and MHC calss II molecules that can be important in T cell migration to epidermis as well as in hapten presentation. In fact, in the elicitation phase, due to the presence of primed T cells in the skin, antigen presentation is not necessary to be done within thy lymph nodes by specialized APC as langerhans cells.

Endothelial cell activation is a necessary process for recruiting inflammatory cells. Activated T cells secrete cytokines that would in

turn modify the expression of adhesion molecules (selectins) in the post-capillary veinules allowing leukocyte migration from the blood vessel to the dermis (29). Once in the dermis, migrate to the superficial dermis and to the epidermis, and induce histological changes typical of contact dermatitis, namely an epidermal oedema leading to the exocytosis of T lymphocytes in the epidermis and the development of vesicles.

The inflammatory reaction persists during only a few days and rapidly decreases due to down-regulating physiological mechanisms.

CD8+T Cells are the major effector cells in CHS

Several lines of evidence support for the major role of CD8+T cells as effector cells in CHS (6,30). This is in contrast to classical DTH that is mediated by MHC class II-restricted CD4+T cells (26).

In a recent study, we have re-examined the subset of T cells involved as effector cells of CHS in mice deficient for either MHC class I-restricted CD8+ cells (β2M-deficient mice), MHC class II-restricted CD4+ cells ($A\beta$-deficient mice) or both (30). Our data clearly demonstrated that CD8+T cells mediated CHS, since i) mice lacking class I molecules and thus depleted in class I-restricted CD8+T cells cannot develop CHS; (ii) MHC class II-knock out mice (deficient in CD4+T cells) which have a compensatory increase in CD8+T cells exhibit exaggerated and prolonged hapten-specific CHS response as compared to normal controls; (iii) In vivo mAb-mediated depletion of CD8+ T cells in class II-knock out mice completely abolished CHS response. The CD8+ cells infiltrate the skin at the site of challenge as really as 18 hours after the epicutaneous application of the hapten and produce locally type 1 cytokines, especially IFNγ.

The fact that the patients with late-stage AIDS (depleted in CD4+ but not CD8+) could still develop allergic cnotact dermatitis, but no tuberculin-type DTH responses further confirm that the human CHS response does follow the same rule as those found in animal models.

Regulation of the contact senitivity reaction: Roles of IL-10 and Hapten specific CD4+ T Cells

The general mechanisms underlying the down-regulation of acute or chronic inflammatory reactions are still not well understood. CHS is a subacute self-limited skin inflammatory reaction. The rapid decrease of the skin inflammation suggests that active mechanisms, including activation of suppressor cells, induction of immunological tolerance or release of inhibitory cytokines, may down-regulate the CHS reaction (31).

IL-10, a potent immunosuppressive cytokine via the inhibition of antigenpresenting cell functiions, has been recently shown to down-regulate CHS as well as most of the classical DTH reactions (32,33). IL-10 is normally secreted by Th2 cells and blocks the IFBγ production by Th1 cells. Mice with targeted disruptions of the IL-10 gene was found to exhibit an enhanced and prolonged CHS response (33). Down-regulation of CHS through the production of IL-10 is probably through activation of either MHC clas II-resticted CD4+T cells (34) or keratinocytes (35) following hapten application. We have already shown that depletion of MHC class II-restricted CD4+T cells would result in an exaggerated and sustained CHS inflammatory response highlighting the important regulatory role of CD4+ cells in CHS (34).

Ongoing studies will undoubtedly provide more insights on how CD4+ regulatory T cells could be specifically activated and thus provide new ways of treating allergic contact drmatitis.

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FIGURE LEGEND.

Schematic Model illustrating the sensitization and elicitation phases of contact hypersensitivity sensitivity.

sensitization phase

Epicutaneous application of hapten induces cytokine secretion by langerhans cells (LC) and keratiocytes which launches activation and migration of Ag carrying LC to the dermis (2) and to the draining lymph nodes through the afferent lymphatic vessels. LC are located in the para-cortical area of the lymph nodes where they can present the processed hapten (haptenated peptides) to specific T cells (●) (step 3), leading to the generation of i) MHC class I-CD8+ T cells that would finally behave as effector cells, and ii) MHC class II-restricted CD4+ T cells that would have a regulatory effect in elicitation phase (⊕). Ag activated T cells emigrate from the lymph nodes and recirculate through the efferent lymphatic vessels and the thoracic duct (step 4). During

this process they acquire the CLA antigen and become memory T cells. CLA+ T cells preferentially infiltrate the skin after trasn-endothelial migration.

Elicitation Phase

When the hapten is applied for a second (and subsequent) time, it diffuses through the epidermis and could be presented by APC to specific T cells in the dermis (step 5). T cell activation is responsible for cytokine production whic induces the activation of other cell types including endothelial cells and keratinocytes (step 6). This results in the production of inflammatory cytokines allowing the recruitment of infiltrating cells (⊘,⊘) from the blood to the dermis and to the epidermis (step 7 and 8).