Update on the pathophysiology with special emphasis on CD8 effector T cells and CD4 regulatory T cells

Abstract: Allergic contact dermatitis (ACD) referred to as contact hypersensitivity (CHS) is one of the most frequent inflammatory skin diseases characterized by redness, papules, and vesicles, followed by scaling and dry skin. ACD is elicited upon skin contact with non-protein chemicals called haptens and corresponds to a cutaneous delayed-type hypersensitivity reaction, mediated by hapten-specific T cells. During the sensitization phase, both CD4+ and CD8+ T cell precursors are activated in the draining lymph nodes by presentation of haptenated peptides by skin dendritic cells (DC). Subsequent hapten painting on a remote skin site induces the recruitment and activation of specific T cells at the site of challenge leading to apoptosis of keratinocytes, recruitment of inflammatory cells and development of clinical symptoms. Experimental studies from the last 10 years have demonstrated that, in normal CHS responses to strong haptenes, CD8+ type 1 T cells are effector cells of CHS through cytotoxicity and IFNg production while CD4+ T cells are endowed with down-regulatory functions. The latter may correspond to the recently described CD4+ CD25+ regulatory T cell population. However, in some instances, especially those where there is a deficient CD8 T cell pool, CD4+ T cells can be effector cells of CHS. Ongoing studies will have to confirm that the pathophysiology of human ACD is similar to the mouse CHS and that the CHS response to common weak haptens, most frequently involved in human ACD, is similar to that reported for strong haptenes.

Keywords: Apoptosis; Dermatitis, allergic contact; Inflammation; Skin

Resumo: A dermatite de contato alérgica (DCA), também conhecida como hipersensibilidade de contato (HSC) é uma das dermatopatias inflamatórias mais frequentes, sendo caracterizada por eritema, pápulas e vesículas, seguidas de ressecamento e descamação. A DCA é induzida pelo contato da pele com substâncias químicas não protéicas denominadas báptenos, e corresponde a uma reação de hipersensibilidade cutânea do tipo tardio, mediada por células T hapténicas-específicas. Durante a fase de sensibilização, tanto os precursores de células T CD4+ quanto os de CD8+ são ativados nos linfonodos de drenagem através da apresentação de pepídeos conjugados a báptenos pelas células dendríticas (CD) da pele. A subsequente exposição de pele ao bápteno em um local a distância induz o recrutamento e ativação de células T específicas no local de provocação, levando à apoptose dos queratinócitos, recrutamento de células inflamatórias e desenvolvimento de sintomas clínicos. Estudos experimentais dos últimos 10 anos demonstraram que, em respostas normais de HSC a báptenos fortes, as células T CD8+ do tipo 1 são efetoras da HSC através de citotoxicidade e produção de IFNg, enquanto que as células T CD4+ são dotadas de funções de regulação negativa. Estas últimas podem corresponder à população de células T reguladoras CD4+ CD25+ recentemente descritas. Entretanto, em algumas situações, especialmente naqueles em que há um pool deficiente de células T CD8, as células T CD4+ podem ser efetoras da HSC. Estudos em andamento terão que confirmar que a fisiopatologia da DCA em humanos é semelhante à HSC em camundongos, e que a resposta de HSC a báptenos fracos comuns, mais frequentemente envolvidos na DCA em humanos, é semelhante à descrita para báptenos fortes.

Palavras-chave: Apoptose; Dermatite alérgica de contato; Inflamação; Pele

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INTRODUCTION

Contact Dermatitis is a frequent inflammatory skin disease in industrialized countries with a great socioeconomic impact and is one of the most common occupational diseases. As the outermost barrier of the human body, the skin is the first to encounter chemical and physical factors from the environment. According to the pathophysiological mechanisms involved, two main types of contact dermatitis may be distinguished. Irritant contact dermatitis is due to the proinflammatory and toxic effects of xenobiotics able to activate the skin innate immunity. Allergic contact dermatitis (ACD) requires the activation of antigen-specific acquired immunity leading to the development of effector T cells, which mediate the skin inflammation. It is characterized by redness, papules, and vesicles, followed by scaling and dry skin.

Allergic contact dermatitis (ACD), also referred to as contact hypersensitivity (CHS), is a T cell-mediated skin inflammatory reaction due to repeated contact of the skin with non-protein chemicals, called haptens. In contrast to classical DTH, which requires intradermal injection of exogenous protein, initiation of CHS is generated by topical application on the epidermis of sensitizing haptens (i.e., nickel, chrome, DNFB, TNCB, oxazolone). Studies from the last 10 years have emphasized that CD8+ T cells are the main effector cells of CHS in all strains of mice. It is noteworthy that there is still some controversy as to whether CD8+ T cells are effector cells of CHS in all strains of mice and for all types of haptens. Similarly, the relative contribution of CD4 and CD8 T cells in human ACD is still unclear. CD8+ T cells are now known to mediate DTH responses in allergic contact dermatitis, drug eruptions, asthma, and autoimmune diseases. The difference between CD8+ T cells and CD4+ T cells mediating DTH may relate to the molecular mechanisms by which antigens are processed and presented to the T cells. Exogenous antigens are phagocytosed and processed on MHC class II molecules (e.g., HLA-DR) for presentation to CD4+ T cells. In contrast, cytoplasmic antigens are processed by the endogenous pathway on MHC class I molecules (e.g., HLA-A, -B, and -C) for presentation to CD8+ T cells. External allergens can also enter the endogenous pathway to be presented to CD8+ T cells. These include many contact sensitizers, chemical and protein respiratory allergens, viral antigens, metabolic products of drugs, and autoantigens. Haptens are also able to directly interact with peptides which are already in the groove of MHC class II and class I molecules.

Thus CD8+ and CD4+ T cells could be activated in the lymph nodes by antigen presenting cells (APC) expressing haptenated peptides presented in the groove of MHC class I and class II molecules, respectively.

1. PATHOPHYSIOLOGY OF CONTACT HYPERSENSITIVITY - General scheme

Knowledge of the pathophysiology of ACD is derived chiefly from animal models in which the skin inflammation induced by hapten painting onto the skin is referred to as contact hypersensitivity (CHS). Two temporally and spatially dissociated phases are usually necessary to achieve optimal CHS reaction: the sensitization and the elicitation phases (Figure 1). We describe here the well-accepted pathophysiological pathways of CHS and ACD. The sensitization phase (also known as afferent phase) occurs at the first skin contact with a hapten and leads to the priming and expansion of hapten specific T cells in lymph nodes. Topically applied hapten is uptaken by skin DC, especially Langerhans cells (LC), which migrate from the epidermis to the paracortical area of draining lymph nodes, where they present haptenated peptide/MHC molecule complexes to hapten-specific T cell precursors. Specific T cells emigrate from the lymph nodes and enter the blood through the thoracic duct and recirculate in the blood and secondary Knowledge of the pathophysiology of ACD is derived chiefly from animal models in which the skin inflammation induced by hapten painting onto the skin is referred to as contact hypersensitivity (CHS). Two temporally and spatially dissociated phases are usually necessary to achieve optimal CHS reaction: the sensitization and the elicitation phases (Figure 1). We describe here the well-accepted pathophysiological pathways of CHS and ACD. The sensitization phase (also known as afferent phase) occurs at the first skin contact with a hapten and leads to the priming and expansion of hapten specific T cells in lymph nodes. Topically applied hapten is uptaken by skin DC, especially Langerhans cells (LC), which migrate from the epidermis to the paracortical area of draining lymph nodes, where they present haptenated peptide/MHC molecule complexes to hapten-specific T cell precursors.
Specific T cells emigrate from the lymph nodes and enter the blood through the thoracic duct and recirculate in the blood and secondary lymphoid organs. The elicitation phase occurs a few hours after a subsequent skin contact with the same hapten which induces chemokine production, endothelial cells and mast cells activation, and neutrophils infiltration, all necessary for recruiting specific T cells. T cells interact with hapten-bearing skin antigen-presenting cells. Activated CD8+ cytotoxic T cells produce type 1 cytokines (IFNγ) and induce skin cell activation and keratinocyte apoptosis leading to the amplification of the cutaneous inflammation through production of a whole set of cytokines and chemokines. These latter allowing recruitment of polymorphous cellular infiltrate characteristic of CHS. This efferent phase of CHS lasts 72 hours in humans and 24 to 48 hours in mice. The inflammatory reaction persists over several days and progressively decreases upon physiological down-regulating mechanisms.

2. CHS TO STRONG EXPERIMENTAL HAPTENS

The vast majority of data available on CHS have been obtained with strong sensitizers, such as DNFB, DNCB, TNP, oxazolone, which have unique chemical and immunological properties: i) they represent a minority of the chemicals among the thousands that are able to induce ACD in humans; ii) they are endowed with potent proinflammatory properties, known as irritancy, due to the toxicity of the chemical. This toxicity provides a danger signal for the skin innate immune system, leading to production of inflammatory cytokines (IL-1, TNF) and chemokines by skin resident cells and to activation of skin DC that can initiate their maturation process and migrate into lymph nodes. DC maturation and migration into lymph nodes is mandatory for initiation of CHS, and several cytokines, chemokines and their receptors, among which CCL21 and its ligand CCR7, are important in this process.11

It is noteworthy that strong haptens can induce a primary ACD, i.e., a hapten-specific
immune reaction, following a single skin contact, which has the same pathophysiology as the classical CHS reaction obtained with two hapten skin paintings.\textsuperscript{12} The occurrence of CHS following a single exposure to haptens could be explained by the persistence of haptens in the skin for several days after painting, allowing the recruitment of specific T cells at the site of skin sensitization. Thus, strong haptens, through their toxicity, deliver danger signals able to potently activate innate immunity that allows the development of a robust and rapid hapten-specific immunity.

Alternatively, the most frequently encountered haptens, classified as moderate, weak or very weak, are much less irritant than strong haptens and may not have the same ability to activate innate immune cells (see chapter 3).

2.1 CD8+ T cells are effector cells while CD4+ T cells behave as regulatory cells

The respective contribution of CD4+ and CD8+ T cells in CHS has been examined using different strategies: i) \textit{in vivo} depletion of normal mice with anti-CD4 and anti-CD8 mAbs; ii) transfer of CD4+ or CD8+ T cells from sensitized mice into immuno incompetent Rag\textsuperscript{2/2} mice; iii) use of MHC class I\textsuperscript{+} (CD8+ T cell-deficient) or MHC class II\textsuperscript{+} (CD4+ T cell-deficient) mice; iv) transfer of hapteneated DC from MHC class I\textsuperscript{+} or MHC class II\textsuperscript{+} mice to induce CHS in naïve recipients. Mice genetically deficient in CD4 or CD8 molecules (CD4\textsuperscript{−/−} or CD8\textsuperscript{−/−}) do not represent relevant models of CD4+ or CD8+ T cell deficiency and results obtained with these mice will be discussed later (see chapter 4 "CD4-deficient mice").

Adoptive transfer experiments first highlighted that DTH to protein was transferable into MHC class II-matched recipients, whereas transfer of CHS required class I-matched recipients.\textsuperscript{15} Using \textit{in vivo} depletion of CD4+ and CD8+ T cell subsets, Gocinski and Tigelaar were the first to suggest that CD8+ T cells could mediate the CHS response to DNFB and other strong haptens.\textsuperscript{14} They further showed that CD4+ T cells were endowed with a down-regulatory activity, since the CHS reaction was enhanced following \textit{in vivo} depletion of CD4+ T cells.

Bour et al. used another approach to study the contribution of CD4+ and CD8+ T cell subsets. They studied CHS in MHC class I and MHC class II KO mice, which are deficient in CD8+ and CD4+ T cells, respectively.\textsuperscript{15,17} Indeed, CD4+ T cells develop during the ontogeny by interaction with thymic APCs expressing MHC class II molecules. Thus, in the absence of MHC class II molecules, thymic precursors cannot differentiate into CD4+ T cell and therefore most of the circulating mature T cells are CD8+ T cells. Likewise, due to lack of positive selection of CD8+ T cells in MHC class I-deficient mice, CD4+ T cells constitute the vast majority of mature peripheral T cells. Surprisingly, class I\textsuperscript{+} mice did not develop any CHS response to DNFB, indicating that CD8+ T cells were mandatory for the development of the disease. Since these I\textsuperscript{+} mice have normal numbers and functions of CD4+ T cells and can mount a classical DTH reaction to alloantigens and to protein antigens,\textsuperscript{12-14} these data demonstrated that CD4+ T cells do not mediate the CHS reaction to DNFB. On the other hand, class II\textsuperscript{−/−} mice developed an enhanced CHS reaction, with chronic skin inflammation, and \textit{in vivo} depletion of CD8+ T cells in these MHC mice resulted in a complete abrogation of the CHS. More importantly, hapten-specific CD8+ effectors could develop in the absence of CD4 T cell help. Indeed, there is no need of CD4+ T cells for the priming of CD8+ T cells in II\textsuperscript{−/−} mice and the presence of CD4+ T cells has a negative effect on the intensity of the CD8+ T cell-mediated CHS response.\textsuperscript{15,16} Thus, other important information from these studies was the characterization of MHC class II-restricted CD4+ T cells as down-regulatory cells of CHS. Most of the data summarized above have been obtained with DNFB in C57BL/6 (H2b)\textsuperscript{15-19} and in BALB/c (H2d) mice.\textsuperscript{20} Similar results have been obtained with other strong haptens such as oxazolone,\textsuperscript{20,21} DMBA\textsuperscript{22} and TNP.\textsuperscript{18,23} Thus, these findings indicated that a functional dichotomy exists between CD8+ T cells and CD4+ T cells which behave as effector cells and regulatory cells, respectively, in CHS to strong haptens.

One of the properties of chemically reactive haptens is their ability to simultaneously generate immunogenic determinants for hapten-specific CD8+ and CD4+ T cells. As discussed later (chapter 5), CD4+ T cells can be effector cells of CHS to TNP in the event of CD8+ T cell deficiency. The reason why effector cells of CHS are only confined in the CD8+ T cell pool in normal mice has recently been explained by Martin S et al.’s studies which showed that CD8+ T cells induce Fas-mediated apoptosis of CD4+ T cells, therefore preventing the priming/expansion of hapten-specific CD4+ T cells during the sensitization phase of CHS.\textsuperscript{24}

2.2 Priming of specific CD8+ and CD4+ in lymphoid organs during the sensitization phase of CHS
CD8+ type 1 cells and CD4+ type 2 cells

The optimal time between hapten painting and T cell priming is 5 days in mice. At that time, T cells recovered from lymph nodes are endowed with potent proliferative activities. Analysis of cytokine production by CD4 and CD8 T cell subsets after in vitro restimulation by haptenated APCs has shown that CD8+ T cells produce type 1 cytokines, mostly IFN-γ, while CD4+ T cells produce type 2 cytokines, including IL-4, IL-5 and IL-10. Analysis of the frequency of DNFB-specific CD8+ T cells by IFN-γ ELISPOT showed an average of 50 CD8+ T cell precursors/10^5 lymph node cells at day 5 post sensitization, a number which is similar to that found in other antigen-specific immune responses.

MHC restriction of hapten specific T cells

Investigators from different groups provided evidence that hapten presentation to T cells in CHS was MHC restricted and thus similar to the presentation of protein antigens in classical DTH. Immunization of mice with hapten-pulsed DC recovered from the epidermis or derived from bone-marrow precursors is able to prime specific T cells that proliferate to DNFB in secondary proliferative responses. Immunization procedures using DC recovered from MHC class I or MHC class II-deficient mice confirmed the opposite functional effects of the CD8 and CD4 T cell pools. In these experiments, MHC class I-expressing DC (either from normal mice or from MHC I+/I- mice) induced the priming of CD8+ T cells in the lymph nodes (assessed by specific proliferation) and the CHS reaction upon subsequent challenge. Conversely, immunization by DC lacking MHC class I molecules (recovered from MHC class I-deficient mice) was inefficient at inducing a CHS reaction but could prime CD4+ T cells. Indeed, CD4+ T cells purified from the lymph nodes of such mice were hapten-specific, as assessed in secondary proliferative responses. These results were confirmed by a recent study in non-genetically modified mice, using bone-marrow-derived DC, which were pulsed with trinitrophenyl (TNP)-derived peptides and administered intradermally to generate a CHS reaction. Two types of peptides that have affinity for either MHC class I or class II peptides were used. Martin et al. showed that class I binding peptides induced CHS responses similar to that obtained with epicutaneous TNP application. In contrast, DC pulsed with class II binding peptides did not sensitize for optimal CHS. On this basis, Cavani et al. speculated that the ability of chemical hapten to drive CD8+ T cell activation might be associated with their capacity to directly bind to self peptides present in the groove of MHC-class I or class II molecules.

CD8+ T cell priming does not require CD4+ T cell help

Classically, optimal activation of naive CD8+ T cells requires signals received by CD4+ T cells, and referred to as CD4+ T cell help. During CHS to strong hapten, CD8+ T cell activation in lymph nodes does not require CD4+ T cell help nor contribution of CD40/CD40-L interaction, since CD40- L-deficient mice mount a normal CHS to DNFB. Mice with CD4+ T cell deficiency induced by either in vivo treatment with anti-CD4 mAb or by genetic disruption of MHC class II genes (MHC class II KO mice) develop a strong CHS response to hapten. The fact that CHS can develop in the absence of class II-restricted CD4+ T cells was further confirmed by the observation that CHS could be induced by immunization with hapten-derivatized DC from class II+/I- mice and with DC from wild type mice pulsed with haptenated MHC class I peptides.

Other studies on viral-induced DTH responses have indicated that activation of naive CD8+ T cells for the generation of MHC class I restricted immune responses can occur in the absence of T cell help. Recently, it was demonstrated that the main parameter dictating the requirement or not of CD4 help was the number of CTL precursors which could be activated at the time of priming. CTL responses induced by cross-priming can be converted from CD4-dependent to CD4-independent by increasing the frequency of CTL precursors. In the absence of CD4 T cells, high numbers of CTL precursors were able to expand and become effector CTLs. The ability of high frequencies of CD8 T cells to override help was not due to their ability to signal CD40 via expression of CD154. These findings suggest that when precursor frequencies are high, priming of CD8 T cell responses may not require CD4 T cell help. Another explanation for the development of CD8+ effector cells is that antigens that have the intrinsic ability to induce DC maturation bypass the need for CD4 help via CD40 activation. Indeed, mice depleted in CD4+ T cells can be primed for CTL responses by transfer of LPS-activated, antigen-pulsed DC. In CHS, DC maturation induced by hapten with strong inflammatory capacities may bypass the need for CD4 help via
CD40/CD40-L interaction and may be sufficient to trigger specific CTL responses with a high precursor frequency.

2.3 Elicitation phase of CHS is due to the recruitment and activation of CD8+ cytotoxic T cells (CTLs)

Since the main function of CD8+ T cells is cytotoxicity, the observation that CHS was mediated by CD8+ cells raised the possibility that cytotoxicity was mandatory for expression of CHS. CD8+ CTLs are effector cells of the immune defense system against viruses and tumors and exert their lytic functions through two main independent mechanisms. The secretory pathway involves the release of perforin and granzymes from cytolytic granules. The non secretory pathway involves interaction of Fas-L upregulated during T cell activation, with the apoptosis-inducing Fas molecule on the target cell.

Although mice deficient in either perforin (p−/−) or Fas-L (gld mutant) were equally capable to develop a normal CHS response to DNFB, and contained hapten-specific CD8+ CTLs able to kill haptenated targets, mice double deficient in both Fas-L- and perforin lack specific CD8+ CTLs and could not develop CHS. This demonstrated that cytotoxicity was necessary for the development of the pathologic process and that one cytotoxic pathway could compensate the absence of the other. The CTLs contribution to the effector phase of CHS was further demonstrated by skin analysis at the site of hapten challenge. Akiba et al. demonstrated that CD8+ T cells could infiltrate the challenged skin as early as 9 hours after skin painting and that the kinetics of CD8 recruitment paralleled appearance of IFNγ transcripts and apoptosis of skin epidermal cells. Double staining for MHC class II and apoptotic cells (TUNEL staining) revealed that keratinocytes were the main target of CTLs. Thus, CD8+ T cells are endowed with in vivo cytotoxic activity and keratinocytes behave as antigen presenting cells during the elicitation phase of CHS. Whether other cell types contribute to in situ activation of hapten-specific CTLs remains unknown. Indeed, hapten rapidly diffuse through the epidermis and could be presented as haptenated peptides by several class I-expressing cell types in dermis including DC, endothelial cells, and mast cells which could contribute to the activation of CD8 effectors recruited in the skin. In this respect, Biederman et al. reported that recruitment of neutrophils via MIP-2 exclusively produced by activated mast cells was necessary for the development of CHS.

Recent studies have brought new insights in the precise steps leading to recruitment of effector T cells in the skin upon hapten challenge. P. Askenase’s team has shown that the elicitation of CHS starts by an innate inflammatory skin reaction peaking 2 hrs after challenge, called CHS-initiating phase, which is followed by the classical DTH-like reaction peaking at 24-48 hrs, referred to as CHS-effector phase. The CS initiation phase is due to hapten binding to specific IgM antibodies produced rapidly after the sensitization by B1-B cells. The hapten-IgM immune complexes activate complement leading to production of C5a which behaves as a chemo tactic factor for T cells. Other chemokines have been implicated in the recruitment of effector cells, among which are IL-8, MCP-1, and RANTES. CXCL-1 (Groα) is produced as early as 30 minutes after challenge and is responsible for the infiltration of the skin by neutrophils, a mandatory step for the recruitment of effector T cells.

Apoptosis is involved in several skin pathologies and is not restricted to CHS. In ACD, several reports have emphasized the existence of apoptotic processes involving the epidermis. More recently, Trautmann et al. have demonstrated that skin lesions of atopic dermatitis (AD) were associated with the occurrence of massive apoptosis of epidermal cells. They also demonstrated that, in AD, memory/effector T cells bearing the cutaneous lymphocyte associated antigen (CLA) and CD45 RO are undergoing activation-induced cell death, skewing the immune response toward surviving Th2 cells. Although the contribution of CTLs in the pathophysiology of AD is not precisely known, these data emphasize that epidermal cell apoptosis is a common feature of eczematous dermatoses and suggest that antiapoptotic drugs could be new therapeutic tools in these diseases.

2.4 CD4+ T cells down-regulate the CHS reaction

Regulatory cells are key actors in maintaining peripheral tolerance and controlling inflammatory responses and have been reported to inhibit the development of autoimmune and allergic immune responses in many experimental models. Three main regulatory CD4+ T cell subsets have been identified: i) antigen-specific Tr1 cell clones which produce high amounts of the immunosuppressive cytokine IL-10; ii) antigen-specific Th2-type CD4+ T
cells which antagonize type 1 T cell effectors characteristic of CHS; iii) and naturally occurring CD4+CD25+ T cells.

CD4+ T lymphocytes behave as down-regulatory cells and most likely regulate both the sensitization and elicitation phases of CHS. The frequency of hapten-specific IFNγ-producing CD8+ T cells in skin draining LNs on day 5 after hapten sensitization is much higher in CD4+ T cell deficient mice than in normal mice, suggesting that CD4+ T cells control the development of the CD8+ T cell pool. It is also possible that CD4+ T cells migrating to the challenge site contribute to the control of inflammation and its resolution. Indeed, in the absence of CD4+ T cells, mice develop a more pronounced and persistent inflammation. Moreover, CD4+ T cells are recruited in the challenged skin several hours after recruitment of CD8+ T cells. Thus regulatory CD4+ T cells may control the magnitude of the CHS by regulating in situ activation and functions of CD8+ CTL effectors.

Limited information is currently available regarding whether a particular subset of regulatory cells is involved in the regulation of CHS. Nickel-specific Tr1 cells (producing high amounts of IL-10) have been cloned from skin lesions of ACD patients suggesting that this subset of regulatory cells might contribute to the regulation of the effector phase of contact sensitivity. Indirect evidence for the implication of CD4+CD25+ cells comes from the observation that IL-2-IgG2b fusion protein inhibited CHS and increased the size of the CD4+CD25+ T cell compartment. Our own data, in the model of CHS to DNFB support a role for CD4+CD25+ regulatory T cells in the control of the skin inflammatory response and in the establishment of oral tolerance to hapten. However, the relative contribution of the regulatory CD4+ T cell subsets in the control of ACD and CHS remains to be clarified.

The mechanisms by which CD4+ T cells control the development of specific CD8 T cells and the magnitude of the CHS reaction are still poorly understood. Recent data by Gorbatchev and Fairchild suggest that CD4+ T cells may regulate the CHS responses by eliminating the hapten-presenting APCs in lymphoid organs during the sensitization phase in a Fas-L dependant mechanism. The consequence of this would be a limited access of CD8+ T cells to MHC class I/hapten complexes and costimulatory signals provided by DC during priming for CHS response.

3. CHS TO COMMON WEAK HAPTENS

In contrast to CHS against strong hapten, there is limited information on the effector cells mediating CHS to moderate, weak, and very weak hapten. The main reason is lack of reproducible animal models for these weak hapten. Indeed, attempts to develop ACD to weak hapten in normal mice have so far failed. Several non mutually exclusive hypotheses could explain why repeated skin contact with weak hapten cannot induce ACD: i) at variance with strong hapten, weak hapten do not bear intrinsic pro-inflammatory properties and therefore cannot deliver danger signals mandatory for activation, differentiation, and migration of skin DC to draining LNs; ii) skin contact with weak hapten generates specific T cells without effector functions due to their low frequency and/or to the weak affinity of their T cell receptor; iii) consequently, CHS to weak hapten may be more sensitive to regulatory/suppressive T cells which could efficiently prevent priming of specific effector T cells.

Strong hapten, e.g. DNCB and oxazolone are toxic molecules able to deliver danger signals to skin cells resulting in tissue inflammation within minutes/hours following skin contact. In contrast, weak hapten are unable to induce skin inflammation at the site of skin contact or may generate a mild skin irritancy only, even when used at high concentrations. We postulate that the pathophysiology of ACD to weak hapten is similar to that of ACD to strong hapten and involves CD8 effectors able to infiltrate the skin in sensitized individuals. Ongoing studies in our laboratory are currently testing this hypothesis.

4. CD4+ T CELLS CAN FUNCTION AS CHS EFFECTORS IN CERTAIN SITUATIONS

Although most recent studies have emphasized that CD8+ T cells are the main effectors mediating CHS, it is possible that CD4+ T cells and other cell types may act as CHS effectors in certain experimental conditions.

4.1 Particularity of some chemicals

Some chemicals, e.g. fluorescein isothiocyanate (FITC) and formaldehyde, have been postulated to provoke preferential type 2 cytokine production by CD4+T cells, which have been shown to mediate the CHS reaction.

4.2 CD4-deficient mice

Kondo et al., and Wang et al.’s studies concluded that CD4+ T cells were effectors of CHS, showing that CHS to DNFB and oxazolone...
was greatly impaired in CD4<sup>−/−</sup> mice, genetically deficient in the CD4 molecule. The reason for the discrepancy between these results and those reported by other investigators most likely relies on important functional differences between CD4 deficient, CD4<sup>+</sup> T cell depleted mice, and MHC class II-deficient mice. Indeed, despite disruption of the CD4 gene, CD4<sup>−/−</sup> mice contain double negative CD4<sup>−</sup>CD8<sup>−</sup> cells exerting the normal functions of CD4<sup>+</sup> T cells found in normal mice. In this respect, efficient thymic maturation of helper T cells has been shown to occur in CD4<sup>−/−</sup> mice. Indeed, CD4<sup>−</sup> TCR<sup>αβ</sup> cells of CD4<sup>−/−</sup> mice do not express the CD4 molecule, they are able to mount MHC class II-restricted responses, suggesting that the CD4 coreceptor is dispensable for efficient recognition of antigens presented by MHC class II molecules on antigen presenting cells.

4.3 CD4<sup>+</sup> T cells may be effector cells in CHS to haptens when CD8<sup>+</sup> T cells are deficient.

CD4<sup>+</sup> T cells could be effector cells in CHS to some haptens when the CD8<sup>+</sup> T cell population is deficient. Evidence came from studies of Martin et al., who first showed that dendritic cells (DC) pulsed with TNP-derivatized peptides that have affinity for class II molecules could induce a low, albeit significant CHS reaction. Next, they used C57BL/6 mice and class I<sup>−/−</sup> mice and studied the CHS to DNP and TNP. CHS to DNP was normal in C57BL/6 mice and absent in class I<sup>−/−</sup> mice, as previously reported. TNP was able to induce a CHS response in C57BL/6 which was inhibited by in vivo depletion of CD8<sup>+</sup> T cells using specific mAbs. Surprisingly, class I<sup>−/−</sup> mice were able to develop a normal CHS reaction to TNP which was abrogated by depletion of CD4<sup>+</sup> T cells.

4.4 Contribution of other cell types in CHS

Besides TCR<sup>αβ</sup> T cells which represent hapten-specific CHS effectors, other lymphoid cell subsets have been shown to contribute to the complex pathway ultimately leading to the CHS response. B-1 cells are activated in lymphoid organs within hours after skin sensitization and produce IgM antibodies. These antibodies diffuse in the skin and will bind the hapten immediately after the challenge. The presence of immune complexes turns on complement activation that seems mandatory for the recruitment of effector T cells at the challenge site. Recent data suggest that T-cell recruitment depends on this early antigen-specific “initiation process” of CHS which is mediated by activation of IL-4 producing liver NKT cells. Epicutaneous immunization causes a rapid and dramatic increase in the percentage of liver iNKT which doubles within 2 hours and remains elevated for up to 24 hours.

Neutrophils play an important role in the development of CHS. In their absence CHS is reduced. From the literature, they are involved in the CHS-initiating and the CHS-effector phase of the disease. Neutrophils are among the first cells to be recruited after challenge of sensitized mice through the chemotactic effect of CXCL-1 (Groα) and they appear before the infiltration of effector CD8<sup>+</sup> T cells. Once effector cells have been activated, another influx of neutrophils is secondary to the activation of mast cells which produce TNFα and CXCL-2 (MIP-2).

CONCLUSIONS

In summary, ACD can be viewed as the result of activation of two distinct T cell subsets endowed with opposite functions: effector T cells and down-regulatory T cells. The severity and the duration of the skin inflammation appear directly related to the respective activation state and/or size of these two compartments. Thus, overwhelming regulation in sensitized individuals may lead to lack of inflammation (tolerance) despite repeated exposures to the hapten, while defects in the number or functions of regulatory cells may explain chronic contact dermatitis. Further studies will have to address the possibility of reversing an established ACD by either targeting the effector T cell population, preventing the recruitment of leucocytes into the skin or increasing the number or functional properties of regulatory T cells.
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1. Haptens are:
   a) low molecular weight chemical substances
   b) electrophilic compounds
   c) able to bind to nucleophilic residues
   d) all of the above

2. Dendritic cells express:
   a) MHC class I molecules
   b) MHC class II molecules
   c) adhesion molecules
   d) all of the above

3. When a contact allergen is applied to the skin, it is uptaken by:
   a) Langerhans cells
   b) T lymphocytes
   c) B lymphocytes
   d) eosinophilic leukocytes

4. Hapten-specific T cells are activated by:
   a) free haptens
   b) extracellular haptenated peptides
   c) haptenated peptides anchored in the antigen-binding grooves of MHC molecules
   d) hapten bound to immunoglobulins

5. Activation of naïve hapten-specific T cells occurs:
   a) in the epidermis
   b) in the dermis
   c) in the draining lymph nodes
   d) in the blood

6. During their migration to the lymph nodes, LC undergo:
   a) morphological modifications
   b) phenotypical modifications
   c) functional modifications
   d) all of the above

7. Haptenated peptides may be expressed on dendritic cell surface as a result of:
   a) direct interaction with peptides anchored in the antigen-binding grooves of MHC molecules
   b) endocytosis of extra-cellular haptenated peptides and expression in association with MHC class II molecules
   c) direct penetration of the cellular wall and expression in association with MHC class I molecules
   d) all of the above

8. Hapten-specific CD8+ T cells are activated through presentation of haptenated peptides by:
   a) MHC class I molecules
   b) MHC class II molecules
   c) co-stimulatory molecules
   d) immunoglobulins

9. The expression of the contact sensitivity reaction involves the activation of:
   a) keratinocytes
   b) endothelial cells
   c) effector T cells
   d) leukocytes migrating in the superficial dermis

10. The inflammatory reaction is down-regulated by:
    a) B cells
    b) granulocytes
    c) T cells producing type 1 cytokines
    d) CD4+ T cells

11. The efferent phase of contact hypersensitivity is characterized by:
    a) occurring few hours after subsequent hapten contact
    b) lasting approximately 72 hours in humans
    c) involving production of chemokines and IFNg
    d) all the above answers

12. The cytotoxic CD8+ cells:
    a) inhibit the inflammatory infiltrate in contact hypersensitivity
    b) produce IFNg and amplify skin inflammation
    c) produce chemokines and inhibit keratinocyte apoptosis
    d) all the above answers

13. Contact hypersensitivity to strong haptens:
    a) involves production of IL-1 and IL-2 by Langerhans cells
    b) occurs by means of chemical toxicity that stimulates CD4+ cells
    c) occurs after activation of innate immune response cells
    d) can not occur following a single exposure
14. In MHC class II KO mice:
   a) the circulating cells are CD8+ with no CHS to strong hapten
   b) the circulating cells are CD4+ with no CHS to strong hapten
   c) there is deficiency of CD8+ cells with CHS to strong hapten
   d) there is deficiency of CD4+ with CHS to strong hapten

15. During the sensitization phase of CHS:
   a) the CD4+ cells produce IL-4, IL-5, IL-10
   b) the Langerhans cells produce IL-12 and several chemokines
   c) the CD8+ cells have no proliferative activity
   d) all the above answers

16. During the elicitation phase of contact dermatitis:
   a) the Langerhans cells produce chemokines that stimulate CD4+ cell
   b) the keratinocytes behave as antigen presenting cells and may be subject to cytotoxicity
   c) cytotoxicity is mainly mediated by CD4+ cells
   d) none of the above answers

17. Recruiting T cells in the skin after hapten challenge:
   a) occurs after activation of complement and production of C5a
   b) involves participation of IL-8, MCP-1 and RANTES
   c) is preceded by neutrophil infiltration
   d) all the above answers

18. Epidermal cell apoptosis:
   a) is described in contact dermatitis
   b) may occur in atopic dermatitis and in other eczemas
   c) could be treated with anti-apoptotic drugs
   d) all the above answers

19. The regulatory activity of CD4+ cells:
   a) occurs by an unknown mechanism
   b) occurs by elimination through cytotoxicity of CD8+ cells
   c) involves eliminating the antigen presenting cells in lymphoid organs
   d) none of the above answers

20. In the future, the therapeutic strategy to control contact dermatitis:
   a) should target the effector T cells
   b) should consider preventing recruitment of leukocytes into the skin
   c) should stimulate the function of regulatory cells
   d) all the above answers

**ANSWERS**

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