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Claudia Traidl-Hoffmann, Hans F. Merk, Andrea Cavani, Nicolas Hunzelmann

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New Insights into the Pathomechanisms of Contact Dermatitis by the Use of Transgenic Mouse Models

Claudia Traidl[a,c] Hans F. Merk[a] Andrea Cavani[c]
Nicolas Hunzelmann[b]

Departments of Dermatology, [a]RWTH Aachen and [b]University of Cologne, Germany;
[c]Istituto Dermopatico dell'Immacolata, IRCCS, Rome, Italy

Abstract

Allergic contact dermatitis (ACD) is one of the most common skin diseases with a great socioeconomic impact. Although extensively studied, its pathophysiology and the interaction of different cells and factors which lead to sensitization and elicitation reaction are still not completely understood. The advent of transgenic mouse technology has considerably changed the study of ACD. This technology has been used extensively to investigate biomedically important issues in such diverse areas as mammalian development, neurophysiology and immunology. This new approach has already led to fascinating results which are confirmatory but also contradictory to previous thinking on the role of certain cytokines, adhesion and cell surface molecules in the complex pathophysiologic process of ACD. This review will describe how recent experiments employing mice carrying cytokine and accessory molecule transgenes change our current understanding of contact dermatitis which may lead to improved therapeutic strategies.

Introduction

Contact dermatitis (CD) by definition encompasses both acute and chronic manifestations of irritant and allergic eczematous diseases. CD is among the most common skin diseases with an estimated prevalence between 6.2 and 10.6% over a period of 1–3 years [Coenraads et al., 1992]. This disease is of major importance in occupational medicine, as in industrialized countries it represents the most common cause of occupational skin disease [Diepgen and Coenraads, 1999]. To classify the principal mode of elicitation, irritant and allergic CD (ACD) are generally discerned. On clinical grounds including histopathology differentiating between these two forms can be difficult, particularly in chronic cases [Lachapelle, 1992]. Irritant CD occurs primarily as a result of chemical toxicity to epidermal cells which then leads to an inflammatory response in the dermis. ACD, in contrast, is the result of a specific immune response towards chemicals penetrating the skin.

Dr. N. Hunzelmann
Department of Dermatology, University of Cologne
Joseph-Stelzmann-Strasse 9, D–50924 Cologne (Germany)
Tel. +49 0221 478 4539, Fax +49 0221 478 4549

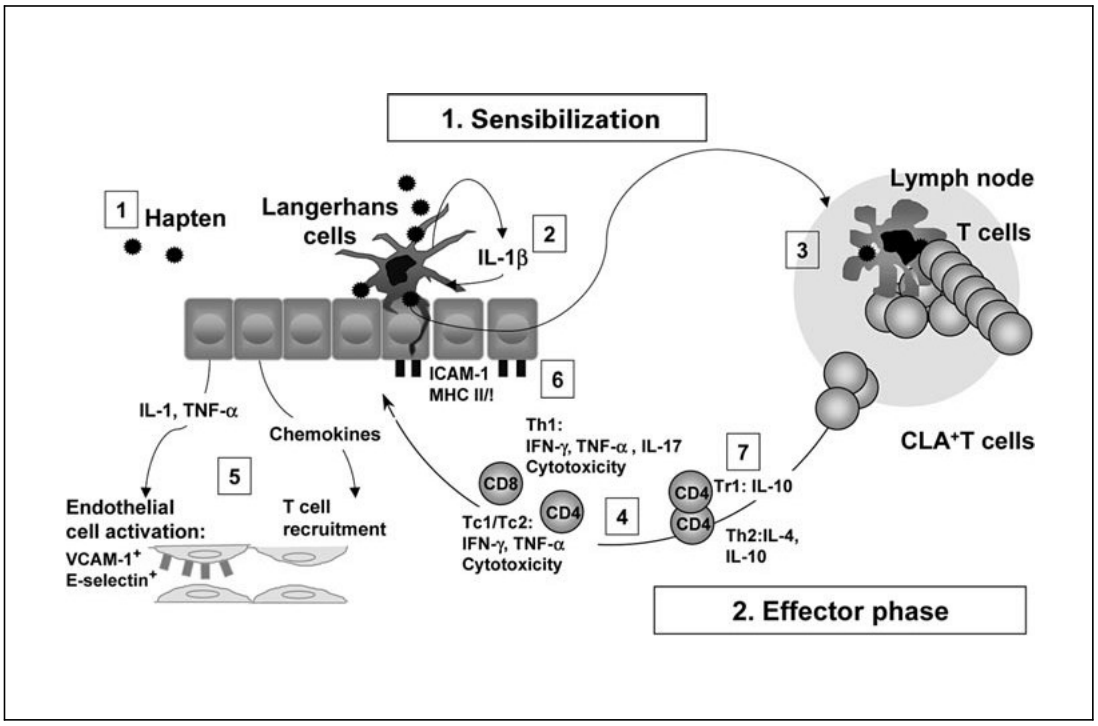

Fig. 1. The dual phase model of contact dermatitis. 1–3 Sensitization phase, 4–7 Effector phase. Haptens (✹) penetrate the stratum corneum (1). Hapten loading by epidermal Langerhans cells parallels activation and migration of Langerhans cells to the draining lymph nodes (2) where they can present haptenated peptides to T cells leading to MHC-class I- or II-restricted allergen-specific CD4+ and CD8+ T cells, respectively (3). The second encounter with the antigen leads to expansion and invasions of CLA+ antigen-specific T cells in the skin – at the side of contact (4). T cell activation is responsible for cytokine production which induces the activation of other cell types including keratinocytes. This results in the production of cytokines and chemokines allowing the recruitment of infiltrating cells (5). Furthermore proinflammatory cytokines such as IFN-γ upregulate MHC-class I and induce MHC-class II and ICAM-1 expression on keratinocytes rendering them susceptible for CD4+ and CD8+ T cell mediated cytotoxicity (6). Regulatory T cells (Tr1) are contributing to the resolution of the inflammatory process (7).

By virtue of the accessibility of the involved organ – the skin – ACD has been in the focus of research trying to elucidate the mechanisms underlying this reaction since the 1930s. Current understanding supports the dual-phase model of CD: the sensitization and effector phase (fig. 1). In the sensitization phase, low-molecular-weight haptens penetrate the skin and are coupled to host proteins – a mechanism called haptenization [Landsteiner et al., 1935; Landsteiner and Chase, 1942]. However, the factors determining the allergenicity of a given molecule are to date not yet fully understood [Basketter et al., 1995]. The modified 'self-proteins' are taken up by resident epidermal dendritic cells (DCs) – the Langerhans cells. Sensitization appeared to depend on an intact route to the local lymph node [Macher and Chase, 1969]. In fact, studies provided evidence for the migra-

tion of Langerhans cells after exposure to haptens into the regional lymph node [Macatonia et al., 1987; Kripke et al., 1990]. Here, antigen presentation leads to the development of antigen-specific T-cell proliferation. The clonally expanded T-cell population gains the propensity to recirculate into the skin via the expression of skin homing factors such as the cutaneous lymphocyte-associated antigen [Picker et al., 1991; Robert and Kupper, 1999]. In the secondary (effector or elicitation) phase of ACD, the re-exposure to the hapten results in antigen presentation by Langerhans cells to memory T cells – in the skin as well as in the regional lymph nodes. This second and every consecutive encounter with the relevant antigen results in a vigorous T-cell-mediated immune response with T cells infiltrating the dermis and epidermis following the inflammatory reaction with the clinical signs of CD. This inflammatory process is self limited, the contraregulatory mechanisms only being poorly understood. Both soluble and cellular factors may be involved in this process. IL-10 is a potent anti-inflammatory cytokine produced by various cell types, including T lymphocytes, monocytes and DCs [de Waal Malefyt et al., 1993; Del Prete et al., 1993], which downregulates immune responses mainly by inhibiting the monocyte- and DC-presenting capacity [Enk et al., 1993a, b; Steinbrink et al., 1997; Morel et al., 1997; O'Farrell et al., 1998]. Recently, in humans a new subpopulation of T cells has been described, which shows a unique cytokine pattern with high production of IL-10 but low or no IFN-γ and no IL-4 which exerts a remarkable regulatory function on DCs and nickel-specific Th1 cells [Cavani et al., 2000]. Keratinocytes are profoundly involved in the regulation of the elicitation and effector phase of ACD, since they secrete cytokines and chemokines which effectively activate resident DCs and endothelial cells [Albanesi et al., 1999] and contribute to lymphocyte recruitment into the skin [Robert and Kupper, 1999]. Conversely it has been shown that keratinocytes also have downregulatory capacities. T-cell activation in the absence of costimulatory signals can lead to induction of anergy [Nickoloff and Turka, 1994]. In fact, Gaspari and Katz [1991] demonstrated that keratinocytes as nonprofessional antigen-presenting cells induce anergy in hapten-specific T cells.

Much of this current dogma pertaining to ACD was developed by performing experiments on specific immunologically relevant cell types isolated either from rodents or humans. However, until recently, the role of cytokines and adhesion molecules in the pathogenesis of CD could only be studied indirectly either unspecifically by the use of pharmacological agents as steroids or UV light or by inhibition of their function through the use of specific antibodies. Studies using antibodies may be potentially misleading as a result of artifacts arising from inappropriate cross-linking events, inadequate penetration in vivo and Fc-receptor-mediated effects.

The use in immunologic research of mice in which selected genes are either expressed (transgenic) or eliminated (knockout mice) has already proven to be a powerful tool towards the elucidation of immunologic mechanisms [Pahram, 1999]. Transgenic animals are created by the injection of DNA containing the desired gene into the pronucleolus of fertilized mouse ova followed by introduction of these zygotes into a pseudopregnant female where they develop into fully transformed transgenic mice [Gordon et al., 1980; Sigmund, 1993]. In knockout animals a disabled version of a gene is created by the introduction of mutations with recombinant DNA methods. The defective gene is introduced into embryonic stem cells, and mutant cells are selected with the use of drug resistance

markers, injected into blastocysts and implanted in receptive females for further differentiation and development. Tissue-specific and inducible promoters allow the expression of the transgenes to be controlled and to be expressed tissue specifically. The human keratin 14 (K14) and K5 promoters have been particularly useful in targeting the expression of transgenes to the mitotically active basal layer of mouse epidermis and to the outer root sheath of the hair follicle, oral epithelia and esophagus [Munz et al., 1999; Sano et al., 1999]. Conversely the K10 and involucrin promoters have enabled targeting of gene expression to the differentiating suprabasal layers of epidermis [Carroll et al., 1993] and a segment of K1 promoter has been useful in targeting expression to all epidermal layers [Greenhalgh et al., 1993]. The generated transgenic mice can be used to investigate the role of a gene of interest in a specific disease process, i.e. contact dermatitis, more in detail. This new approach has already delivered fascinating results which are confirmatory but also contradictory to previous understanding concerning the role of certain cytokines and accessory molecules in ACD. Therefore this review aims to describe how recent experiments using this new immunotechnology deepen our current understanding of the pathogenesis of CD and thus may offer new opportunities to develop new therapeutic approaches to this complex disease process.

Overview of Cytokine Transgenic Mice in the Study of Contact Dermatitis

The role of several cytokines which are presumably an important element in the development of CD has been studied in transgenic mice either by overexpressing or deleting the gene. Th1 cells producing IL-2 and IFN-γ are thought to play an eminent role in the evolution of contact hypersensitivity (CHS) as it could be shown that these cells could passively transfer this reaction [Hauser, 1990]. Mice overexpressing human IL-2 in the skin driven by the MHC H-2b class I promoter develop a generalized dermatitis, alopecia and erythema [Akiyama et al., 1993]. These changes are accompanied on a microscopic level by an increased number of perivascular lymphocytes and a diffuse infiltration of the skin by neutrophils and monocytes. Transgenic mice expressing IFN-γ driven by the involucrin promoter in the epidermis show an increased and persistent CHS reaction to topical application of 2,4-dinitrofluorobenzene (DNFB) [Carroll et al., 1997]. In contrast, IFN-γ-deficient mice [Goes et al., 1995] reveal a diminished local skin inflammation and HLA-DR expression following induction of the CHS response. Furthermore, these mice lack the marked systemic MHC class I and II induction seen in wild-type mice. Interestingly other authors revealed that CHS in sensitized animals was not significantly reduced by the disruption of the IFN-γ gene. However, dermal mononuclear infiltrates and epidermal microabscesses were clearly diminished in the mutant mice. Thus, it is concluded that CHS is partially dependent on a functional IFN-γ system. While the cutaneous edema is IFN-γ independent, the mononuclear cell infiltration and epidermal microabscess formation are at least partly IFN-γ dependent [Saulnier et al., 1995].

On the other hand, overexpression of IL-6 in the epidermis driven by a human K14 promoter [Turksen et al., 1992] surprisingly did not result in inflammatory changes in the dermis or epidermis, as e.g. leukocytic infiltration, but is followed by an increased stratum corneum whereas epidermal proliferation and differentiation remain unaffected. These observations suggest that IL-6 does not exhibit a

pronounced proinflammatory activity in the skin and does not cause inflammation by itself.

IL-1β has been attributed an important role in the early phase of the induction of CD [Enk and Katz, 1992]. Thus, it is of interest that conflicting results have been obtained on the importance of IL-1β during the early phase of contact sensitization. While expression analysis of IL-1β in BALB/c mice [Enk and Katz, 1992] as well as the use of neutralizing antibodies [Enk et al., 1993a] suggested an essential role for IL-1β during CHS, no difference in the induction and elicitation response towards oxazolone was found in mice of mixed C57/BL6J and 129Sv(ev) background rendered deficient in IL-1β [Zheng et al., 1995]. This may partly be due to blocking antibodies eliciting a more complex reaction. In addition, this result in IL-1β-deficient mice may also indicate that other cytokines may compensate for the loss of IL-1β. The assumption of a compensatory mechanism is further supported by Shornick et al. [1996]. These authors found that in a different strain of mice deficient in IL-1β using oxazolone as the antigen, the degrees of ear swelling were indistinguishable in wild-type and IL-1β-deficient mice. In addition, using lower doses of sensitizing antigen (in this case trinitrochlorbenzole, TNCB) CHS was shown to be defective but could be restored using high doses of the sensitizing antigen.

Studies aimed at defining the role of T-helper 2 (Th2) cells provided evidence that these cells are inactive or may even exert suppressive effects in CHS reactions [Cher and Mosmann, 1987; Asada et al., 1997]. This view has been supported by a study evaluating CHS in C57BL/6 mice with a targeted disruption of the IL-4 gene showing no difference in the response to oxazolone from wild-type mice [Berg et al., 1995]. Nevertheless, several in vivo and in vitro studies indicate that IL-4 apart from suppressive might also have proinflammatory properties [Müller et al., 1993; Röcken et al., 1996; Salerno et al., 1995]. To determine the role of IL-4 in the generation and regulation of CHS in the BALB/c strain, we treated BALB/c mice lacking the IL-4 gene with dinitrochlorobenzene (DNCB) to induce CHS. Here, the mutant mice lacking the IL-4 gene showed a marked attenuation in magnitude and duration of the CHS compared to normal syngeneic mice (44% ± 12% of normal at 24 h after challenge assessed by net ear swelling; $p < 0.05$) [Traidl et al., 1999]. This attenuation was accompanied by a marked reduction of cellular infiltrates in the dermis and reduced epidermal necrosis when compared to wild-type mice.

Mutant mice also showed hyporesponsiveness in inflammatory reactions to irritant concentrations of DNCB suggesting that IL-4 is also required for optimal induction of irritant CD. Interestingly, adoptive transfer experiments revealed that no CHS response could be elicited when lymph node cells from sensitized IL-4-deficient mice were injected into the ears of control mice. This indicates that IL-4 is an important constituent of the cytokine milieu during the early phase of CHS, as it has been suggested by studies of Salerno et al. [1995]. However, using oxazolone as the allergen, no significant difference in ear swelling was observed. These results suggest that still undefined properties of the chemical compound used to elicit the CHS response play an important role in the regulation of the cytokine network during the allergic reaction. The differential response to the different allergens used, i.e. DNCB or oxazolone, is similar to the results obtained with the IL-1β-deficient mice [Shornick et al., 1996; Zheng et al., 1995]. This indicates that the mechanism underlying the hypersensitivity responses to TNCB, DNCB and oxazolone may partly evolve on different pathways be-

ing dependent and independent of IL-1β and IL-4, respectively.

Further hints to a proinflammatory role of IL-4 were given analyzing CHS responses in mice deficient in signal transducer and activator of transcription 6 (STAT6) [Yokozeki et al., 2000]. The CHS response was significantly reduced in STAT6(–/–) mice compared with wild-type mice. The expression of Th2 cytokines (IL-4, IL-5) in TNCB-challenged skin tissues and the supernatants from hapten-specific T cells were profoundly reduced in STAT6(–/–) mice, whereas the expression of IFN-γ was the same in STAT6(–/–) and wild-type mice after challenge. These data suggest that the STAT6 signal for the production of IL-4 and IL-13 plays a critical role in the induction of CHS and underlines the results pointing to a proinflammatory role of IL-4.

Transforming growth factor β1 (TGF-β1) regulates leukocytes and epithelial cells. Interestingly, TGF-β1, a pleiotropic cytokine known to have also immunosuppressive properties, appears to be a cytokine essential for epidermal localization and development of murine Langerhans cells [Borkowski et al., 1996]. I-A+ Langerhans cells could not be detected in epidermal cell suspensions prepared from TGF-β1(–/–) mice. Further experiments showed that the Langerhans cell defect in TGF-β1-deficient mice is not due to an epidermal abnormality but reflects a requirement of murine Langerhans cells (or their precursors) for TGF-β1 [Borkowski et al., 1997].

Early studies in the knockout mouse model revealed a downregulatory role of IL-10 in CHS response. Berg et al. [1995] demonstrated that mice deficient for IL-10 showed an exaggerated immunoresponse to haptens. Recent studies further investigated the role of IL-10 in CD pointing to an enhanced epidermal Langerhans cell migration in IL-10-deficient mice indicating that IL-10 plays an inhibitory role in Langerhans cell migration. Proofs were given that this effect may occur via the downregulation of TNF-α and IL-1 production [Wang et al., 1999a]. Also mechanisms of therapeutic effects could be elucidated using the knockout mouse model. Simkin et al. [2000] showed that IL-10 is as a key modulator in the inhibition of the CHS response by whole-body photodynamic therapy (PDT) with verteporfin. They demonstrated that DNFB-sensitized mice given PDT with verteporfin and whole-body red light irradiation exhibited a significant reduction in CHS compared with animals without treatment. Administration of rIL-12 reversed the effect of PDT as did treatment of mice with anti-IL-10-neutralizing antibody. Notably, knockout mice deficient for IL-10 were found to be resistant to the inhibitory effects of PDT [Simkin et al., 2000].

Overview of the Use of Transgenic Mice of Cell Surface Molecules in the Study of CD

Several accessory molecules are also induced during the sensitization and elicitation phase of ACD and therefore may play a significant role in the progression of the disease. A large number of adhesion molecules has been shown to be an important element in the pathogenesis of CD. Also several molecules involved in antigen presentation, such as major histocompatibility complex molecules or co-stimulatory molecules, are thought to be essential in ACD [Murayama et al., 1997]. On keratinocytes and in the perivascular infiltrates of the CHS response, a marked induction of the intercellular adhesion molecule 1 (ICAM-1) is observed. The pathogenetic relevance of this observation is confirmed when ICAM-1-deficient mice are examined for their ability to generate a CHS response [Sligh

et al., 1993]. Mutant mice exhibited a 74% suppression of CHS reflecting a mostly absent edema and mononuclear cell infiltrate in the skin when compared to sensitized wild-type animals. However, overexpression of this molecule in the epidermis [Williams and Kupper, 1994] driven by the K14 promoter did not potentiate CHS reactions indicating that ICAM-1 does not act independently in the elicitation of cutaneous inflammation.

Costimulatory signals which are not antigen specific nor MHC restricted play an important role in T lymphocyte triggering [Springer, 1990]. The B7 antigens (B7-1, B7-2) and the contrareceptors CD28 and CTLA-4 expressed on T cells are crucial for the induction of IL-2 production and in preventing anergy induction. Transgenic mice whose epidermal keratinocytes driven by the K14 promoter express high levels of cell surface B7-1 antigen show unchanged histological appearance when compared to wild-type mice [Nasir et al., 1994]. However, these mice develop an exaggerated and persistent cutaneous delayed-type hypersensitivity reaction. This indicates that overexpression of B7-1 interferes with the role of keratinocytes in the resolution of the delayed-type hypersensitivity reaction. Additionally, CHS to DNFB and oxazolone but also to irritants as e.g. croton oil was significantly decreased but not abolished in CD28(–/–) mutant mice [Kondo et al., 1996b]. Significant increases in IL-2 mRNA were detected in the skin after DNFB challenge in normal mice, while this response was blunted in CD28(–/–) mice. In addition, the results of this study suggest that ACD and irritant dermatitis have overlapping pathophysiologic mechanisms, but subtle differences exist.

Even though early studies pointed to Th1 cells as the major effector cells of CHS, recent results are pointing to CD8+ T cells being mainly responsible for the effector mechanisms whereas CD4+ T cells seem to be effector and regulator of the immune response of CHS [Gocinski and Tigelaar, 1990; Bour et al., 1995; Bouloc et al., 1998]. This idea is in line with the fact that mice lacking MHC class II show increased CHS responses [Bouloc et al., 1998] pointing to regulatory CD4+ T cells. However, mutant mice lacking the CD4 molecule [Kondo et al., 1996a] showed marked hyporesponsiveness in ACD and irritant CD suggesting that the CD4 molecule is required for optimal induction of CHS as well as irritant CD and may influence the development of CHS by modulating the cytokine profiles in the skin. Kehren et al. [1999] aimed at defining the role of cytotoxic mechanisms in CHS and could demonstrate that $P^{0/0}$ *gld* mice lacking Fas ligand (FasL) and perforin genes, both involved in T-cell-mediated cytotoxicity, fail to mount CHS reactions. The central role of CD8+ T cells and the importance of FasL and perforin-mediated cytotoxicity by nickel-specific T cells in human CD has recently been demonstrated [Traidl et al., 2000].

The recruitment of T cells into the skin is a crucial step in the development of CHS. Adhesive interactions play an important role in the mechanism by promoting leukocyte attachment and extravasation from the vasculature into the peripheral tissues. T cells positive for cutaneous lymphocyte-associated antigen of different antigen specificity represent 10–15 % of all circulating T cells in peripheral blood and are known to be the skin-homing T cells [Robert and Kupper, 1999]. They gain the capacity to recirculate into the skin via the expression of cutaneous lymphocyte-activated antigen and its ligation to selectins expressed on activated endothelial cells [Picker et al., 1991]. Experiments investigating the role of endothelial cell L- and P-selectin expression underline the importance of this compartment for the cutaneous immune response demonstrating that the recruitment of

T cells in the dermis is impaired in P- and *d*-L-selectin-deficient mice [Subramaniam et al., 1995; Tedder et al., 1995]. Recently it could be demonstrated that some haptens apart from cytokines may upregulate adhesion molecules such as P- and L-selectin on endothelial cells via the NF-κ B pathway [Goebeler et al., 1993, 1995].

Noninflamed skin venules support constitutive leukocyte rolling – a process preceding the transmigration of T cells into the skin. P-selectin controls the rolling frequency, whereas E-selectin dictates rolling velocity. Fucosylated selectin ligands are essential for all interactions, as rolling was absent in mice doubly deficient in α1,3-fucosyltransferase (FucT) IV and FucT-VII. The rolling fraction was reduced in FucT-VII–/– animals but normal in FucT-IV–/– mice. However, rolling velocity was markedly increased in both strains. These results demonstrate a role for FucT-IV in selectin-dependent adhesion and suggest that the endothelial selectins and FucTs have distinct but overlapping functions in the immunosurveillance of the skin [Weninger et al., 2000]. Mice deficient for the major collagen-binding integrins, α1β1 and α2β1, showed a reduced CHS response. This result underlines the importance of the expression of these molecules in the leukocyte infiltration and edema formation in CHS response [De Fougerolles et al., 2000]. There is also growing evidence that β2-integrins have an impact on the migration of mononuclear cells into sites of inflammation as shown by the CD18 knockout mouse model [Walzog et al., 1999].

One of the most dramatic influences on DCs is provided by CD40 stimulation in vitro [Caux et al., 1994; Bjorck et al., 1997], raising the possibility that in vivo, some DC functions might be dependent on CD40-CD40 ligand interactions. As discussed above CHS is a T-cell-mediated response dependent on intact DC functions. Results from CD40L-deficient mice reveal that CD40-CD40 ligand interactions in vivo play a key role in regulating the migration of antigen-bearing epidermal DCs to drain lymph nodes and subsequently the initiation of an acquired immune response. They also imply that an important outcome of these interactions is the production of cutaneous TNF-α, a known mediator of epidermal DC migration. Moodycliffe et al. [2000] conclude that CD40-CD40 ligand interactions might be considered as a functional bridge between innate responses and the initiation of acquired immunity.

The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, whose physiologic functions include tissue remodeling and embryogenesis by degradation of most of the components of the extracellular matrix. The importance of this group of proteins in the processes of tumor invasion and metastasis is now widely acknowledged. MMPs are also expressed by T cells and macrophages, but there is a paucity of evidence for their role in CHS responses. Studies in mice with deficiencies of stromelysin 1 (MMP-3) or gelatinase B (MMP-9) in a DNFB-induced model of CHS showed that stromelysin-1-deficient mice develop an impaired CHS response to topical DNFB. An intradermal injection of stromelysin 1 immediately before DNFB sensitization rescued the impaired CHS response to DNFB in stromelysin-1-deficient mice. In contrast, gelatinase-B-deficient mice exhibited a persistent CHS response. Interestingly, gelatinase-B-deficient mice failed to show IL-10 production at the site of CHS, an essential feature of resolution in control mice. These results indicate that stromelysin 1 and gelatinase B serve important functions in CHS. Stromelysin 1 is required for initiation of the response, whereas gelatinase B plays a critical role in its resolution [Wang et al., 1999b].

Future Aspects of the Use of Transgenic Mice in Contact Dermatitis Research

After the introduction of the term 'transgenic' by Gordon et al. [1980] to describe the successful integration of foreign DNA into the mouse genome by pronuclear microinjection of fertilized mouse oocytes, this system has revolutionized immunologic research and has proven to be the method of choice for scientists to study the role of a gene in vivo. The results presented above using transgenic technology to study the pathophysiology of CD led to important new results and insights of the role of different adhesion molecules and cytokines in the disease process. However, due to the complexity of the development of CD and the pleiotropic functions of cytokines on immune cells, different, in part even contrary results have been obtained in transgenic mice. This suggests that a thorough understanding will rely on significantly more refined methods than just inducing or inhibiting the function of a gene in the whole organism.

To gain more insight in the factors involved in the mechanisms of ACD, the rather coarse experiments on the role of cytokines and accessory molecules in ACD performed recently with mice having lost or gained function of a gene in all compartments during their whole life span will soon be followed by experiments aimed at specific cell subsets at specific time points. In fact, although the use of transgenic mouse technology has greatly advanced our understanding of epidermal protein function, there are numbers of serious limitations to these strategies. In some cases for instance, a gene may perform a function that is a key to epidermal growth, but germline mutations of the gene may cause early embryonic lethality. Efficient silencing of a specific gene in a temporally regulated and epidermis-specific fashion could extend functional analyses to broadly expressed genes. In this light new transgenic methods were developed. Yet, recent developments leading to a marked refinement of transgene technology allow to influence gene expression in a tissue-specific, i.e. cell-specific way by the use of specific promoters, as it is for the skin, e.g. the K14 promoter. Inducible ablation or induction [Munz et al., 1999; Sano et al., 1999] as well as the induction at a given time point by the use of a promoter exclusively sensitive to an exogenously added drug as for example tetracycline or tamoxifen [Vasioukhin et al., 1999; Legname et al., 2000].

In addition, an issue only beginning to be addressed is the role of the genetic background, as it is observed that the phenotype of a mutation may differ depending on the strain of mice in which it is analyzed. The analysis of differences in the background of genes interacting with the mutated gene will be another exciting future aspect.

An increasingly useful strategy for effecting conditional gene inactivation or activation in mammalian systems employs the Cre recombinase of phage P1, the so-called Cre/lox system. In bacteria, the 38-kD Cre protein catalyzes an efficient site-specific recombination between 34-bp sites called *loxP*. Remarkably, Cre also catalyzes an efficient DNA recombination in vivo at *loxP* sites placed into the genomes of yeast, plants and mammalian cells (first described by Sauer [1987]). Thus, a DNA segment flanked by directly repeated *loxP* sites can be excised from the genome in a Cre-dependent fashion, and this strategy has been used in mice to activate expression of a target gene, delete an endogenous gene and to remove the selectable marker that remains after homologous targeting in embryonic stem cells. Spatial and temporal control of excision events can be achieved by imposing the desired control on either the expression or activity of Cre

recombinase. Similarly, the related yeast site-specific DNA recombinases FLP and R have become useful in manipulating the genomes of higher eukaryotes (reviewed in Sauer [1998]). These tools now pave the way for testing the functional role of any cytokine or accessory molecule at specific time points and specific tissue sites. An extension of the above-mentioned techniques is the combination of the bacterial recombinase technique with adenovirus-based technology, which would open vast possibilities of tissue-specific genetic modifications in a controlled time frame.

The xenobiotic-metabolizing enzyme system cytochrome P450 (CYP) is involved in the metabolism of many drugs, regulating their plasma concentrations and activities. The impact of this enzyme system on the metabolization of allergen is up to now poorly understood, but in vitro studies are pointing to its importance for some allergens [Hahn et al., 1993]. The use of a P450 knockout mouse model, targeting different P450 isoenzymes, as it has already been described [Buters et al., 1999; Kimura et al., 1999], could be a useful tool to understand the importance of allergen metabolization in the development of CHS.

The technology for manipulating mouse genetics is, as shown above, advancing rapidly and knockouts of a gene of interest are already offered on a commercial basis and legal disputes related to new transgene techniques are under way. Yet it has to be kept in mind that the mouse immune and inflammatory repertoire is different from the human. Thus, a transfer of results obtained in the murine to the human system has to be done with caution which may impair the impact of studies in mice on the human system.

Furthermore, despite intense research in the field, it is yet impossible, at least on a histological and immunohistochemical level, to discriminate between the allergic and irritant type of CD. Nevertheless, the transgene technology has opened up exciting new ways to study the role of single molecules in the pathogenesis of CD as the paradigm of the delayed-type hypersensitivity reaction which will certainly lead to the development of new therapeutic options.

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