

SPECIAL ARTICLE

# The Immunology of Nickel-Induced Allergic Contact Dermatitis

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Nickel is one of metals widely used for commercial and medical purposes; yet, it is undoubtedly a potential contact allergen.<sup>1</sup> Indeed, nickel allergy is perhaps the relatively commonest type of metal-induced allergic contact dermatitis and the incidence of this contact allergy has doubled during the last 10 years.<sup>1,2</sup> However, the exact mechanisms by which nickel-induced hypersensitivity occurs remains unclear.

The skin is equipped with all cells and cytokines necessary for the generation of an immune response.<sup>3</sup> For example, skin-derived dendritic type cells, such as Langerhans cells (LCs), are able to process and present antigens to CD4<sup>+</sup> cells.<sup>4</sup> Moreover, a recent hypothesis on the mechanism of delayed type hypersensitivity (DTH) responsiveness<sup>5</sup> has led to redefine the concept of the immunopathogenesis of allergic contact dermatitis. The aim of this paper is to briefly review the immunology of nickel-allergic contact dermatitis (NACD) and an attempt to establish a model of the induction of this allergic disorder is made herein.

## T cell regulation of NACD

In normal skin, almost 90% of

**SUMMARY** Attempts have been made to elucidate the immunopathogenesis of contact allergy; yet, the exact mechanism by which nickel-induced allergic contact dermatitis (NACD) occurs is far from clear and is discussed herein. It seems to suggest that a direct nickel-MHC class II molecule binding on the skin antigen presenting cells such as Langerhans cells (LCs) would result in Th1 cell activation. Substances such as serotonin and cytokines such as TNF-alpha produced by activated mast cells may increase adhesion molecule expression and thus, enhance T cell trafficking in the skin. Cytokines such as IFN-gamma and IL-1 and perhaps IL-12 certainly play a crucial role in the activation of Th1 cells. Along with possible function of CD8 cells, downregulation of NACD may be mediated by suppressed function of LCs via the action of activated keratinocytes-derived IL-10. Inhibition of NACD can also be generated by feeding with nickel, suggesting that the induction of oral tolerance to nickel may be beneficial for an alternative immunotherapy of nickel allergy. Nevertheless, this testable model provides a direction for further investigation.

total T cells are CD4 cells. Interestingly, the majority of CD4 cells are the activated cells which express HLA-DR and IL-2 receptor.<sup>6</sup> Indeed, immunohistological studies revealed that in skin biopsies of the NACD patients, most of the CD4 T cells are the activated cells which infiltrate mainly the perivascular and dermal area.<sup>7-11</sup> These findings suggest that antigen-specific CD4 cells play a crucial role in NACD.

Nickel-specific T cell clones derived from peripheral blood and dermal tissues of nickel-sensitive patients were established.<sup>12-16</sup> Characteristically, most of the clones

were phenotyped as CD4<sup>+</sup> T cells and only few were CD8<sup>+</sup> T cells. The majority of the clones recognized nickel in a MHC class II restricted manner. Interestingly, two of the CD4 cell clones isolated from the inflamed tissues were non MHC

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restricted and required no antigen presenting cells (APC) to proliferate.<sup>14</sup> The extrapolation of these findings in NACD remains to be determined. Yet, it may indicate, to some extent, that nickel could act as a direct signal transducer as demonstrated by aluminium fluoride on T cells<sup>17,18</sup>

Of interest, CD4 cell clones derived from nickel sensitive patients produced either IL-2 or IFN-gamma.<sup>12,16</sup> Based on the cytokine production, two murine CD4 cell subsets are defined; thus, CD4 type 1 (Th1) cells produce IL-2 and IFN-gamma, whereas type 2 (Th2) cells are characterized by their ability to produce IL-4, IL-5, IL-6, IL-10 and IL-13 in absence of IL-2 and IFN-gamma (Fig. 1).<sup>19,20</sup> These

CD4 cell subsets are regulated reciprocally and shown to exist in the human immune system.<sup>21</sup> Unlike in the murine immune system, both human IL-10 and IL-13 are released by both Th1 and Th2 cells.<sup>20,22</sup> Moreover, Th1 and Th2 cells are known to mediate DTH response and help antibody production, respectively.<sup>19</sup> Nickel-specific T cell clones producing IL-2 and IFN-gamma correspond therefore to murine Th1 type cells and function as the effector cells in NACD.<sup>12,16</sup> Preferential activation of Th1 cells in human tuberculin reaction supports the notion.<sup>23</sup>

Using contact sensitizers such as oxazolone and picryl chloride in a murine model, DTH response is regulated by two different T cell subsets.<sup>24</sup> DTH-initiating cells are phenotyped as Thy-1<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>-</sup>, CD3<sup>-</sup>, CD5<sup>+</sup>, whilst the DTH-effector cells are CD4<sup>+</sup> cells (see also ref. 5 and 25 for details). These findings have raised the question whether NACD is also mediated by two different T cell subsets. Since nickel-specific T cell clones were isolated from relatively well established NACD patients, no NACD-initiating T cell subset might have been defined.

The role of CD8 cells in NACD is unclear. A recent study has shown that nickel-specific alpha beta TcR-bearing CD8<sup>+</sup> T cell lines could be established from peripheral blood mononuclear cells of patients with nickel allergy.<sup>26</sup> In this study, functional characteristics of these cell lines have not yet been determined. It was suggested however that preferentially expanded nickel-specific CD8 cells seen in an *in vitro* system may reflect the *in vivo* situation in which this antigen-specific T cell subset proliferates more than CD4 cells, following nickel exposure.<sup>26</sup> Indeed, in the animal models, only *in vivo* depletion of both CD4 and CD8 cells but not either CD4 cells or CD8 cells alone was able to

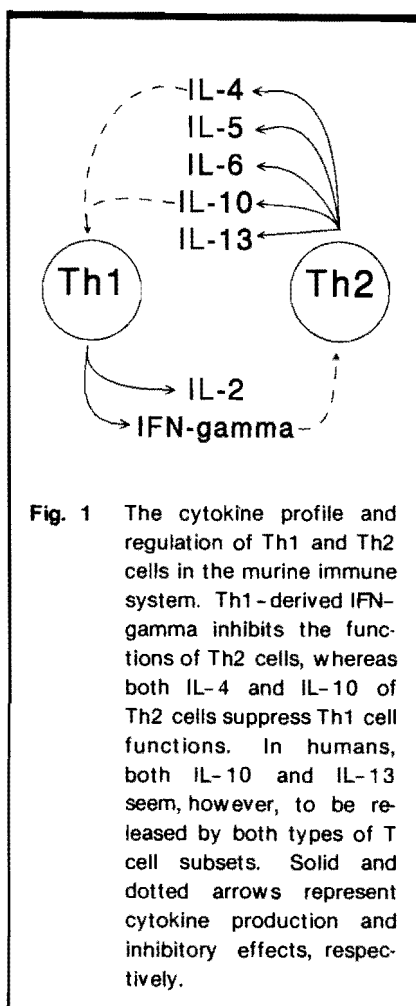
suppress the induction of contact dermatitis,<sup>27</sup> suggesting that both cell subsets are involved in this phenomenon. One may assume therefore that nickel-specific CD8 cells participate in the induction of NACD via the production of certain cytokines such as IL-2 and IFN-gamma. Yet, the role of this cell subset in down-regulation of NACD cannot be ruled out as seen in a study showing that urushiol-specific CD8 cells may down-regulate the induction of contact allergy (see also below).<sup>28</sup>

#### The antigen presenting cells (APC) in NACD

Being recognized by T cells, antigens must be processed and presented as small fragments on MHC molecules by APC. Several types of skin professional APC such as LCs, veiled cells, intermediate cells, tissue macrophages and other non-dendritic type cells have been reported.<sup>3</sup>

An increased expression of LC surface antigens such as MHC class II molecules on the contact dermatitis suggests that this type of APC plays a prominent role in NACD.<sup>29,30</sup> Indeed, nickel-specific T cell activation would be enhanced if co-cultured with irradiated LCs.<sup>13,31,32</sup> LCs are also able to convert naive to antigen-specific T cells which then mediate the contact allergy.<sup>33</sup> Similarly, specific T cells derived from *in vivo* hapten-primed mice were able to produce large amount of IL-2, but not IL-4 in the presence of LCs.<sup>34</sup> Altogether, these results demonstrate therefore that LCs are the primary APC to activate Th1 cells in NACD.

The ability of other professional APC such as keratinocytes within the skin in NACD cannot be ruled out. For example, nickel is able to bind directly on keratinocytes and upregulate the IL-1 production and ICAM-1 expression of these cells.<sup>35</sup> These cells also express MHC class II molecules, which are inducible by IFN-gamma, and



**Fig. 1** The cytokine profile and regulation of Th1 and Th2 cells in the murine immune system. Th1-derived IFN-gamma inhibits the functions of Th2 cells, whereas both IL-4 and IL-10 of Th2 cells suppress Th1 cell functions. In humans, both IL-10 and IL-13 seem, however, to be released by both types of T cell subsets. Solid and dotted arrows represent cytokine production and inhibitory effects, respectively.

enable to activate T cells in tuberculin reaction and leprosy.<sup>36-38</sup> Yet, it seems that in NACD, the role of keratinocytes is to down regulate the contact allergy, since they appear in the relatively late phase of the allergic reaction.<sup>39</sup> In this respect, a support can be drawn from the fact that hapten-pulsed keratinocytes but not LCs induced suppression of contact allergy.<sup>40</sup> These cells may thus be a complementary to LCs in NACD.<sup>41</sup> Additionally, the proliferation of nickel-specific T cells was considerably enhanced by macrophages,<sup>42</sup> but this finding has not yet been further verified.

The exact mechanism by which presentation of nickel by APC to induce specific T cell activation occurs is far from clear. Treatment of epidermal cells with contact sensitizers immediately induced suppression of membrane MHC class II molecules, perhaps due to internalization of molecules into the endocytotic compartments.<sup>43,44</sup> Subsequently, upregulated surface expression of these molecules occurred after 24 hours,<sup>45</sup> suggesting that antigen presentation of contact allergens requires the process of endocytosis and intracellular interaction of endocytosed allergens and MHC class II molecules. However, it may be unlikely to occur in NACD, since the works of Romagnoli and colleagues have in this respect shown that nickel could bind directly to the APC via MHC class II molecule-bound histidine region of self peptides and could thus generate nickel-specific T cell proliferation.<sup>46</sup> These results suggest therefore that the endocytotic process of nickel by APC may be unnecessary.

#### The role of mast cells

That activated mast cells which release various potent substances play a decisive role in the immediate hypersensitivity is well established. Along with these substances, the

ability of mast cells to produce various cytokines highlights the role of this cell population in the induction of not only immediate hypersensitivity but also DTH response.<sup>5, 47,48</sup> Briefly, an antigen-specific IgE-like factor produced by DTH-initiating T cells activates mast cells to release serotonin and TNF-alpha (see also ref. 49). Serotonin increases subsequently vascular permeability, whereas TNF-alpha induces ELAM-1 expression on the endothelial cells, resulting in T cell extravasation from the vascular system. Following antigen recognition, these vascular-originated T cells develop into Th1 effector cells. In humans, skin mast cell degranulation occurred at 1 hour after hapten application and increased ELAM-1 expression could be subsequently observed at 2 hours,<sup>50</sup> suggesting that mast cells indeed play a crucial role in the induction of contact allergy and in T cell migration *in vivo*. Whether cutaneous DTH response induced by nickel is orchestrated by recruiting mast cells to develop CD4 effector cells has not yet been reported. It seems reasonable to believe that mast cells, to some extent, involve in the immunopathogenesis of NACD.

Of interest, recent findings have revealed that mast cells derived from murine bone marrows and rat peritoneal cavity are capable of presenting antigens to T cells in MHC class II restriction.<sup>51,52</sup> However, whether the antigen presentation function of this cell population *in vivo* occurs is unknown. It should be noted that mast cells produce cytokines such as IL-4, which in turn direct the activation of Th2 cells.<sup>19</sup> If mast cells as APC are indeed involved in NACD, this cell population may also function in the late phase of NACD, by inhibition of nickel-specific Th1 cell activation via cytokines such as IL-4 and/or IL-10 (see also below). This contention remains speculative and awaits however to be investigated further.

#### The role of cytokines

The bidirectional communication between cells of the immune system mediated by cytokines is prerequisite. A complex interaction among cytokines in the generation and maintenance of the skin immune system has been reviewed elsewhere.<sup>53,54</sup> Indeed, in the early events of contact sensitivity, LC-derived IL-1 beta, keratinocyte-derived TNF-alpha, IL-1 alpha, protein 10 and MIP-2 as well as T cell-derived IFN-gamma were all upregulated as early as 15 minutes after murine skin painting with allergens.<sup>55</sup> IL-8 was detected as early as half hour after antigen application.<sup>56</sup> Increased ETAF/IL-1, epidermal-derived lymphocyte chemotactic factors, and keratinocyte-derived IL-6 were also observed in the relatively late phase of NACD.<sup>57,58</sup>

Injection of rTNF-alpha enhanced hapten-induced immune response, whereas injection of anti-TNF-alpha antibodies suppressed this response in mice,<sup>59,60</sup> suggesting that this cytokine plays a crucial role on increased contact allergy. It may be that the effect of cytokine in this allergic disorder is attributed to its ability to enhance ICAM-1 and ELAM-1 expressions which in turn determine the T cell trafficking in the skin.<sup>49,61</sup> Despite the fact that increased expression of this cytokine could be detected in the early phase of contact allergy, the exact function of this cytokine in NACD is not known. Yet, it seems plausible that the immunoregulatory role of this cytokine in the expression of adhesion molecules would, at least in part, support the role of this cytokine in NACD.

The role of IL-1 in NACD has not been well defined. A direct interaction of nickel and keratinocytes induced the production of low but significant levels of IL-1.<sup>34</sup> Possibly, IL-1, particularly IL-1 beta, enhances the production of other epidermal-derived cytokines

such as MIP-2 and TNF-alpha and the antigen presentation function of LCs.<sup>62</sup> Furthermore, it is not impossible that T cell-derived IFN-gamma may act as the second signal for mast cell activation which in turn releases other peptides to recruit T cells as discussed above.<sup>5</sup> IFN-gamma produced by Th1 cells is able to inhibit the development of Th2 cells;<sup>19</sup> therefore, increased dermal IFN-gamma following nickel challenge suppresses Th2 cells, allowing the development of Th1 effector cells in NACD.

Previous studies have shown that injection of anti-IL-10 antibodies prior to antigen challenge resulted in prolonged hapten-induced contact allergy, whereas injection of rIL-10 prevented the induction of this immune response in mice.<sup>63,64</sup> The mechanisms by which IL-10 alters the elicitation of this allergic disorder have been believed to associate with reduced IL-1 and TNF-alpha production and decreased antigen presentation function of LCs which in turn induces Th1 cell anergy.<sup>64,65</sup> Direct evidence to implicate the role of IL-10 in NACD has not yet been reported. In fact, murine keratinocytes are shown to be the main source of IL-10 mRNA in the skin.<sup>66</sup> It seems plausible therefore that the suppressive effect of keratinocytes in NACD may be due to its ability to induce downregulation of LCs cells via the production of this cytokine.

IL-2 produced by activated APC is believed to be a powerful polypeptide in the generation of Th1 cells.<sup>67,68</sup> Since human keratinocytes are capable of releasing IL-12,<sup>69</sup> it is thus worthy to determine whether this cytokine is involved in the development of NACD.

#### The role of adhesion molecules

Following antigen challenge, leucocytes interact each other to form cell aggregate and subsequently undergo cell activation as well as

migration. This phenomenon is rapid and mediated by adhesion molecules such as ICAM-1.<sup>70</sup> The role of adhesion molecules in the skin immune system has been shown elsewhere.<sup>71</sup> For example, the expression of ELAM-1, VCAM-1 and ICAM-1 were all enhanced in the early phase of NACD.<sup>72-74</sup> Following nickel-keratinocyte binding *in vitro* enhanced expression of ICAM-1 on keratinocytes could be observed,<sup>35</sup> implying that lymphocyte adherence on the endothelial cells and lymphocyte extravasation from the vascular system in NACD are virtually upregulated. The cell-cell contact in the skin is also increased, enabling to effectively generate the NACD-effector cells. The expression of these molecules certainly provides some insights into T cell recruitment during the nickel-specific DTH responsiveness.

Of interest, anti-ICAM-1 antibodies abrogated the proliferation of KLH-specific Th1 cell, but not Th2 cell clones induced by murine epidermal LCs,<sup>75</sup> suggesting that expression of this adhesion molecule is required to induce Th1 cell-mediated immune response. Thus, it would not be surprising if the optimal expression of this adhesion molecule providing costimulatory signals for stimulation of nickel-specific Th1 cell activation by LCs in NACD is prerequisite. Of note, dendritic type cells express a recently described adhesion molecule called B7/BB1.<sup>76</sup> This molecule has been shown to bind to its ligand, designated as CD28 molecules which are expressed on naive T cells; thus, B7-CD28 binding would result in the activation of naive T cells.<sup>77</sup> These findings suggest therefore that B7 molecules are also a powerful costimulator in the cell activation. Whether these molecules play a role in the induction of NACD is worthy to determine.

#### The genetic background of NACD

The immune system is known

to be under control of genes encoded within the MHC gene set. With respect to NACD, the genetic background in developing nickel sensitivity is still controversy. In a murine model, the development of nickel-induced DTH responsiveness is determined by I-A region of H-2 genes.<sup>78</sup> In humans, HLA class I and II expressions fail to show convincingly their relationship with susceptibility to develop NACD.<sup>79,80</sup> A promising result is perhaps demonstrated by using HLA class III polymorphism (BF, C4a and C4b).<sup>81</sup> It was found that only BF F allele was significantly expressed in the NACD patients (relatively risk = 2.61), suggesting that this gene product involves in the pathogenesis of NACD. The exact role of this increased molecule expression *in vivo* remains to be elucidated; however, it may serve as the nickel-binding serum protein which forms an hapten-protein complex.<sup>81</sup>

#### Oral tolerance and NACD

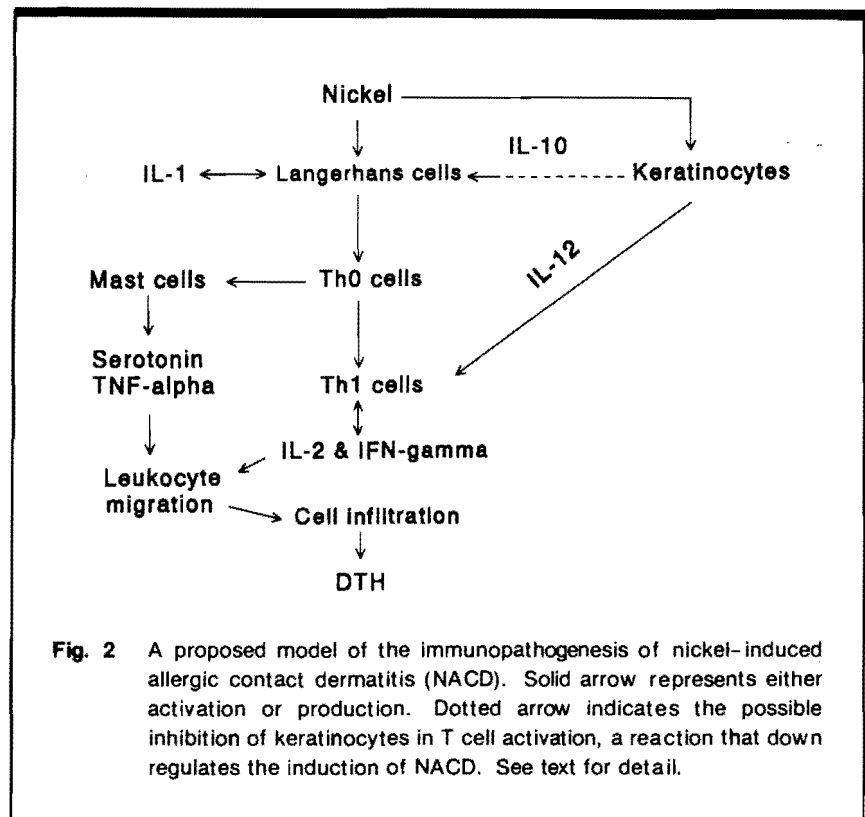
Both oral and nasal immunization with antigens such as protein antigens and allergens have long been known to induce specific immune tolerance following systemic immunization with the same antigens; this phenomenon is termed oral tolerance.<sup>82,83</sup> The precise mechanisms by which this phenomenon occurs seems to depend upon distinct factors such as the types and doses of antigens; thus, both active immune suppression mediated by either suppressor T or B cells and passive immune suppression mediated by T cell anergy have been proposed. Regardless of the mechanisms, the use of this phenomenon as an immunotherapy for certain diseases such as autoimmune diseases in both human and animal studies seems to be promising.<sup>82,83</sup>

Oral immunization of nickel in guinea pigs, but not mice, was able to suppress DTH responsiveness following cutaneous applica-

tion of the same antigen but not chromium, suggesting that specific oral tolerance to nickel occurs.<sup>84,85</sup> Further studies revealed that this immune tolerance was long lasting and mediated by suppressor cells.<sup>86</sup> Of interest, oral tolerance failed to occur if animals were systemically sensitized with nickel prior to oral administration of this contact allergen, suggesting the immune status of subjects determines the induction of oral tolerance and that nickel-specific effector T cells are not susceptible to be orally tolerized.<sup>87</sup> Clinically, these findings based upon the animal model are relevant, since oral challenge with nickel in patients with nickel hypersensitivity induced flare-up reaction and the clinical symptoms were worsened.<sup>11,88,89</sup> However, this increased reaction may be dependent upon the frequency of orally administered nickel. For example, flare-up reaction would occur if patients were given 5 mg of nickel as a single dose,<sup>11,88,89</sup> whereas the same dose of nickel given orally once a week for 6 weeks would result in hyposensitization.<sup>90</sup> This contention is supported by the fact that the induction of nickel allergy could be diminished in patients wearing nickel-containing orthodontic appliances,<sup>91</sup> suggesting that prolonged oral exposure of nickel in these patients may alter the induction of this contact allergy. Notwithstanding, although further studies both in humans and the animal model are required, oral tolerance to nickel may be beneficial as an alternative immunotherapy for NACD.

#### The induction of NACD: a model

The DTH reaction following nickel sensitization is well known, but its precise mechanism is largely unclear. Yet, accumulating evidences show that antigen specific Th1 cells are the effector cells. Despite heterogeneity types of APC in the skin, dendritic-type cells are



**Fig. 2** A proposed model of the immunopathogenesis of nickel-induced allergic contact dermatitis (NACD). Solid arrow represents either activation or production. Dotted arrow indicates the possible inhibition of keratinocytes in T cell activation, a reaction that down regulates the induction of NACD. See text for detail.

likely to be the main cells to process and present the antigen in NACD. The dermal cytokines and adhesion molecules unequivocally regulate the course of NACD. One may speculate therefore that similarity between the classical DTH response as described by Askenase<sup>5</sup> and NACD exists and raises a model of NACD (Fig. 2). Briefly, skin-penetrating nickel would bind to the self peptide-bearing MHC class II molecules on the APC, mainly LCs, which in turn present the antigen to the NACD-initiating T cells. If this is the case, mast cell activation induced by T cell-derived IgE-like factor results in serotonin and TNF-alpha release. Subsequently, increased vascular permeability and lymphocyte-endothelial cell interaction by both polypeptides stimulate T cell extravasation to the allergen-penetrated sites where infiltration of these cells is microscopically evident. The involvement of IL-8 and MIP-2 as chemotactic peptides in the recruitment of naive CD4

cells to this site cannot be ruled out at this stage.<sup>92,93</sup> Following antigen presentation carried out by nickel plus MHC class II-bearing APC, the naive CD4 cells are then converted to antigen specific Th1 effector cells following the action of IFN-gamma and perhaps IL-12. Th1 cell-derived IL-2 and IFN-gamma ultimately induce nonspecific inflammatory cell extravasation which in turn develops perivascular infiltrate, a characteristic of the classical DTH.

This simplified model of NACD does not necessarily omit the possibility that failure of immune tolerance to suppress the course of NACD exists. No significant difference between the functionally active CD8 cells in nickel-sensitive and non-sensitive persons has been observed,<sup>94</sup> suggesting that the development of NACD may be associated with failure of CD8 cells to downregulate the NACD-effector cells. Although the phenotypic cells

are not yet known, orally tolerized suppressor T cells are indeed capable of inhibiting the induction of NACD in the animal models.<sup>68</sup> Alternatively, suppression of NACD may be initiated by keratinocytes via the release of IL-10 as previously discussed. Yet, the exact mechanisms by which keratinocytes could be activated and subsequently acted as a suppressor APC in the late phase of NACD remain questionable. One possibility is that continuous nickel exposure would convert keratinocyte-mediated immune enhancement into immune suppression. This unique behaviour of epidermal APC can be seen from the fact that repeated cycles of hapten-stimulated T cell proliferation by LCs induced the appearance of Th2 rather than Th1 cells. Thus, the first cycle of co-cultured hapten-specific T helper cell lines and LCs resulted in the production of IL-2 detected from the culture medium.<sup>34</sup> In these studies, the third and fourth one have generated high levels of both IL-2 and IL-4; however, only IL-4, but not IL-2 could be detected at the fifth one. By analogous, one may speculate that the initial keratinocyte activation following primary nickel exposure results in the production of IL-2 which in turn augments the Th1 cell activation; subsequently, these cells release IL-10 to down regulate the activation of this helper T cell subset via suppressed function of LCs. A support can be drawn from a comparison between the time course of both keratinocyte-derived IL-10 and IL-12 mRNA expression after haptens applied. The peak levels of IL-10 and IL-12 mRNA expression are reached at 12 and 6 hours respectively,<sup>65,69</sup> suggesting that IL-12 produced by these cells would play a role much earlier than IL-10 in the course of contact allergy.

One of the possible clinical implications of the immunological changes during the course of NACD

is its usefulness as a diagnostic tool in testing the effectiveness of the drugs. For example, topically applied cyclosporin A (CsA) reduced the skin patch test response up to 22% of the nickel-sensitive patients.<sup>95</sup> In this study, T cell infiltration could be diminished, but MHC class II and Leu 6 expression remained intact in CsA-treated patients.

### CONCLUSION

Considerable efforts have been made in elucidating the immunopathogenesis of nickel-induced allergic contact dermatitis (NACD). It appears that following nickel challenge, the skin immunocompetent cells such as T cells and APC and related accessory molecules are all activated to form a complex network resulting in the antigen-specific cutaneous delayed hypersensitivity. There are however some questions, which warrant further investigation, such as the exact role of mast cells, NACD-initiating T cells, CD8 cells, the types of APC, cytokines and adhesion molecules such as B7/BB1 *in vivo*. The advancement of immunotechniques should enable to elucidate the enigmatic NACD in the near future.

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