Dendritic Cells as a Tool for the Predictive Identification of Skin Sensitisation Hazard

The Report and Recommendations of ECVAM Workshop 51[1,2]

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Preface

This is the report of the fifty-first of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM). The main objective of ECVAM, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences, and which *reduce*, *refine* or *replace* the use of laboratory animals. One of the first priorities set by ECVAM was the implementation of procedures that would enable it to become well informed about the state of the art of non-animal test development and validation, and of opportunities for the possible incorporation of alternative methods into regulatory procedures. It was decided that this would be best achieved through a programme of ECVAM workshops, each addressing a specific topic, and at which selected groups of independent international experts would review the current status of various types of *in vitro* tests and their potential uses, and make recommendations about the best ways forward.

The workshop on dendritic cells as a tool for the predictive identification of skin sensitisation hazard was held at ECVAM (Ispra, Italy) on 7–8 April 2004, with participants derived from academia, national organisations, and industry. The aim of the workshop was to review the state of the art of the use of cultured dendritic cells for the identification of skin sensitisation hazard, and to develop strategies for the eventual replacement of *in vivo* testing. At the end of this report are listed the recommendations that should be considered for progressing toward the prevalidation and validation of relevant and reliable procedures, that could in the future replace the use of animals in the skin sensitisation testing of chemicals and cosmetic products.

Introduction

Skin sensitisation resulting in allergic contact dermatitis is an important health issue. Many hundreds of chemicals are known to have the potential to cause skin sensitisation (albeit with widely varying potencies), and there is a need to understand the likely risks to human health. The first step in a

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risk assessment process requires the accurate identification of hazard.

Currently, the identification of skin sensitising chemicals is most commonly achieved by using animal test methods. Included among these are guinea-pig tests, such as the occluded patch test of Buehler (1) and the guinea-pig maximisation test (GPMT; 2), and the mouse local lymph node assay (LLNA; 3). However, there is growing interest in exploring whether it might be possible to design and develop new approaches that would permit an assessment of skin sensitising activity without the need for experimental animals. To this end, attention has focused on a variety of strategies that comprise both the investigation of novel *in vitro* experimental systems, and the possible application of structure–activity relationships and computer-based prediction methods (4–7). Among the approaches that have attracted most interest are those that make use of cultured dendritic cells (DCs) and dedicated antigen processing and presenting cells (7).

The objectives of this workshop were to review the current status of, and future opportunities for, the use of cultured DCs as a basis for the development of alternative approaches to the identification of skin sensitisation hazard. In addition, several specific issues were addressed by the workshop participants, including: the most appropriate DCs for use in *in vitro* skin sensitisation testing; the readouts that may be available for evaluating the interaction of chemicals with DCs; and how putative *in vitro* test methods based on measurement of DC responses might best be integrated into a tiered testing strategy. The progress made during the workshop is summarised here, and deliberations about opportunities for *in vitro* test methods are preceded by a brief consideration of the basic mechanisms of skin sensitisation and of the roles played by DCs.

Skin Sensitisation and DCs

Skin sensitisation is acquired when a (susceptible) subject is exposed topically to a skin sensitising chemical under conditions that will permit the induction of a cutaneous immune response of a magnitude sufficient to result in immunological priming (sensitisation). A key requirement is that the dose of chemical experienced on the skin, and in reality the amount of chemical per unit area of skin, is sufficient to provoke the degree of priming (quantity of response) and quality of response that is required for clinical sensitisation (i.e. a degree of sensitisation that will result in a clinically discernible cutaneous reaction following subsequent encounters with the same chemical).

The sequence of events can be summarised briefly as follows. For immunological recognition, and for the stimulation of an immune response, a skin sensitising chemical must usually form a stable association with protein. It is believed that, in most instances, the hapten–protein conjugate is recognised and internalised by Langerhans cells (LCs), which are specialised DCs found within the epidermis. These cells, under the regulation of cutaneous cytokines, are mobilised and induced to migrate from the skin via afferent lymphatics to regional lymph nodes, where they locate within the paracortex. During migration, LCs are subject to a functional differentiation (also effected by cytokines), such that they lose the ability to internalise and process antigen, and instead acquire the properties of mature immunostimulatory DCs that are able to present antigen effectively to responsive T lymphocytes. Allergen-specific T lymphocytes are activated and stimulated to divide and differentiate. Cell division results in the selective clonal expansion of allergen-responsive T lymphocytes, and this represents the cellular basis for immunological memory and the acquisition of skin sensitisation. If the now-sensitised subject subsequently encounters the same (or an immunologically cross-reactive) chemical allergen, at the same or a different skin site, an accelerated and more aggressive secondary immune response will be elicited. Cells from the expanded population of allergen-responsive T lymphocytes localise at the site of allergen contact, and stimulate the recruitment of other leukocytes that, in concert with secreted cytokines and chemokines, initiate the local inflammatory reaction that is recognised clinically as allergic contact dermatitis. More-detailed reviews of the cellular and molecular immunobiology of skin sensitisation and allergic contact dermatitis are available elsewhere (8–12).

There has been considerable interest in the roles played by LCs during the induction phase of skin sensitisation, and in the molecular mechanisms through which their phenotypic and functional characteristics are regulated. Comprehensive accounts of LC biology in general, and of the importance of LCs in the acquisition of skin sensitisation in particular, are available in the literature (13–19). Collectively, the data available reveal that epidermal LCs become mobilised in response to skin trauma (including encounter with antigen in skin tissues, local irritation and inflammation, ultraviolet [UV] irradiation and, probably, even physical trauma), with the key cytokine signals being tumour necrosis factor α (TNF-α), and interleukins 1 (IL-1α and/or IL-1β) and 18 (IL-18). The successful migration and localisation of LCs is also dependent on chemokine receptor–ligand interactions, with ligation of CCR7 (a chemokine receptor expressed by activated LCs as they progress to draining lymph nodes) being of particular importance.

The LCs within the epidermis can be considered to be sentinels of the adaptive immune system, with

responsibility for sampling skin surfaces for changes in the antigenic microenvironment. Although the view is that the triggering of LC activation requires appropriate danger signals (proinflammatory cytokines; 20), in addition to experiencing new antigens *per se*, a case can nevertheless be made that effective skin sensitisation will be associated with, and dependent upon, the interaction between chemical allergens and resident LCs. Support for this view derived initially from studies reported by Enk & Katz (21), who found that skin sensitisation in mice resulted in the altered expression of the mRNAs for a number of cytokines. Of particular interest was the observation that skin sensitisation, but not similar treatment with a skin irritant, was associated with a very rapid (within 15 minutes) increase in the expression of the mRNA for IL-1β (21), a cytokine that in murine epidermis is a product exclusively of LCs. One interpretation was that chemical allergens interact directly and selectively with epidermal LCs to provoke the increased expression of IL-1β. The implication was that this might form the basis of a method for the identification of chemicals with skin sensitising potential, and this opportunity was articulated in a previous report from ECVAM (22). Progress that has been made to date with this approach, and the tools that are now available to facilitate this, are reviewed in the next section.

The History of Interest in DCs for Sensitisation Studies

In 1992, Enk & Katz (21) showed that mRNA for IL-1β is rapidly expressed by LCs after the topical application of contact sensitisers to mouse skin. More important was the observation that rapid up-regulation of IL-1β mRNA was apparently specific for contact sensitisers. These data, together with other observations, strongly suggested that IL-1β plays an essential role in the induction of cutaneous immune responses, including skin sensitisation (23–25). Thus, it was hoped that IL-1β expression could provide an alternative read-out for assessment of the sensitising activity of chemicals.

Using DCs isolated from human peripheral blood mononuclear cells (PBMC) and differentiated with cytokines, as surrogates for LCs, Reutter *et al.* (26) investigated the ability of five contact sensitisers and one skin irritant, to induce IL-1β gene expression *in vitro* in PBMC-DCs. The DCs were treated with subtoxic concentrations of contact sensitisers (including pentadecyl-catechol, nickel sulphate [$NiSO_4$]) and with the irritant, sodium dodecyl sulphate (SDS). Total RNA was extracted from the DCs, and IL-1β mRNA expression was measured by using the reverse transcriptase-polymerase chain reaction (RT-PCR). The authors reported that all the contact sensitisers increased IL-1β gene expression, whereas treatment with SDS was without significant effect.

Subsequently, Pichowski *et al.* (27, 28) confirmed that potent contact sensitisers selectively up-regulate IL-1β mRNA expression. However, they also observed that, with 2,4-dinitrofluorobenzene (DNFB), such increases were found only with approximately 50% of donors. The variation in DNFB-induced up-regulation of IL-1β expression was shown to be donor-dependent and not due to interexperimental variation. Importantly, the variations among donors with respect to allergen-induced changes in IL-1β mRNA appeared to reflect stable differences, since, on repeat testing, individual donors were found to display consistent responder or non-responder phenotypes. They also confirmed that SDS did not increase IL-1β mRNA levels, even in DC populations that proved responsive to DNFB with respect to IL-1β expression. De Smedt *et al.* (29) also reported that it was difficult to use IL-1β as a reliable marker, due to substantial interindividual variations. Collectively, these data suggest that some chemical allergens have the potential to cause a modest increase in IL-1β expression under conditions where some non-sensitising skin irritants do not. Unfortunately, the fact that blood-derived DCs from only a proportion of donors have a responder phenotype, suggests practical difficulties in ensuring the availability of consistently responsive cells. Therefore, using IL-1β expression does not appear to represent a viable stand-alone method for the identification of skin sensitising chemicals. Moreover, even with the use of an optimal concentration of strong contact allergens in DCs isolated from responders, the observed increases in IL-1β mRNA expression are modest, suggesting that this gene may display a limited dynamic range.

Although expression analysis of IL-1β alone does not look promising at this stage, it is interesting (and of considerable potential importance) to note that some contact allergens appear to interact, probably directly, with cultured DCs, to induce changes in gene expression under conditions where non-sensitising skin irritants apparently do not. Of particular interest would be the identification of genes that are regulated selectively by contact allergens and that display a greater dynamic range of expression than does IL-1β, for example.

Sources of DCs

LCs represent the principal antigen presenting cells in the skin. However, obtaining a sufficient number of cells to be used routinely in *in vitro* assays for skin sensitisation testing remains extremely difficult, despite the availability of various isolation techniques. For this reason, research has focused on other sources of suitable antigen presenting cells.

CD34+-derived DCs

The CD34+ cell fraction comprises committed haematopoietic precursor cells that are capable of differentiating into DCs. Major sources of CD34+ precursors are bone-marrow and umbilical cord blood, since the percentage of CD34+ precursor cells in adult peripheral blood is very low, unless bone-marrow cells are mobilised into the circulation with either growth factors or chemotherapy, or both (30). TNF-α is a critical growth factor for CD34+ progenitors, promoting the *in vitro* proliferation of precursor cells through the induction of the granulocyte/macrophage colony stimulating factor (GM-CSF) receptor (31, 32). In addition, DC yield can be improved by adding FLT3-ligand, thrombopoietin, and stem cell factor (SCF) to the culture medium, which enhances CD34+ cell proliferation without affecting their differentiation (33, 34). At least two populations can be distinguished among CD34+-derived DCs (CD34-DCs), where CLA+ = cutaneous lymphocyte antigen: a CLA+/Birbeck granule+ LC-like DC population, that express E-cadherin and langerin, and a CD1a+/CLA- population that lack Birbeck granules but express coagulation factor XIII, thus resembling interstitial DCs. Both these subsets express major histocompatibility (MHC) class II molecules, together with CD40 and CD54, and moderate levels of CD80 and CD86, consistent with an immature phenotype. In the absence of further activation factors, CD83 can be moderately expressed on a minor percentage of CD34-DCs, and this is greatly increased upon stimulation. The relative percentages of CD1a+/CLA- DC and CD1a+/CLA+ LC populations are affected by the presence in the culture medium of TGF-β, which considerably expands the latter population, supporting the notion that TGF-β is required for LC development both in the human and in the mouse (35). Functionally, CD34-DCs possess equal or higher antigen presenting cell ability than monocyte-derived DCs (Mo-DCs; 36).

Monocyte-derived DCs

Homogeneous populations of immature DCs (resembling those found in peripheral tissues) can be generated by culturing peripheral blood CD14+ monocytes in medium supplemented with GM-CSF and IL-4 (37). These cells are characterised by high-level endocytic activity mediated by constitutive macropinocytosis and by the mannose receptor (38). Mo-DCs express several toll-like receptors (TLR) and respond to microbial stimuli and inflammatory cytokines by up-regulation of MHC and co-stimulatory molecules, down-regulation of endocytic activity and production of chemokines and cytokines. IL-12 production is elicited by some, but not all, microbial stimuli and is potently boosted by interferon (IFN)-γ and especially by CD40L (39). By changing the cytokine cocktail, it is possible to drive monocytes to differentiate to DCs that display different functional properties. GM-CSF, together with type I IFN, induces monocytes to rapidly differentiate toward DCs that are more mature than those induced by GM-CSF and IL-4 (40). Furthermore, the addition of TGF-β to GM-CSF and IL-4 promotes differentiation to DCs that share some characteristics with LCs (41).

Experimental Studies with DCs

LC cultures

As previously described, the important role of LCs in the induction of skin sensitisation justifies the exploration of their usefulness as an *in vitro* model system for the assessment of chemical allergens and irritants. Hitherto, research has mainly focused on induced changes in membrane markers expression and cytokine production.

A dose-dependent decrease of human leukocyte antigen-DR (HLA-DR) expression on the surface of LCs following treatment with several contact allergens, without affecting the number of positive cells, has been reported. In addition, a down-regulation of E-cadherin expression, as well as a significant decrease in the percentage of E-cadherin positive cells, was observed (42). SDS did not significantly affect either HLA-DR or E-cadherin expression. Distinct differences in patterns of HLA-DR endocytosis induced by contact sensitisers and skin irritants in LCs have also been reported (43). Isolated LCs from skin explants showed up-regulation of CD86, CD54 and HLA-DR markers after challenge with a variety of contact allergens, but not following treatment with SDS, and no clear-cut results were obtained for intracellular IL-1β production (44). The results described here have illustrated the feasibility of developing LC-based *in vitro* assays for discriminating between contact sensitisers and skin irritants. However, research has been hampered by the low LC yields available from skin samples and the sometimes spontaneous maturation of LCs during the extraction procedures. For this reason, freshly isolated LCs have mostly been replaced by the use of LC-like DCs (LLDCs) cultured from PBMCs (37) or from CD34+ progenitor cells (30) derived from cord blood or bone-marrow samples.

Monocyte-derived DCs

For chemical sensitisation testing, the *in vitro* model based on Mo-DCs from human peripheral blood is the most frequently used approach. The reasons for this include the shorter culture-times,

the less expensive techniques needed to derive Mo-DCs, and easier access to peripheral blood than to cord blood or bone-marrow samples for the generation of CD34-DCs. In several studies, the effects of sensitisers and irritants on the modulation of surface markers, mRNA expression and cytokine production have been investigated.

Degwert *et al.* (45) and Becker *et al.* (46) reported an increased receptor-mediated endocytosis of HLA-DR in Mo-DCs exposed to several strong contact allergens. Less-active skin sensitisers and skin irritants failed to have a significant effect on HLA-DR internalisation (46). The authors proposed this approach as an *in vitro* test for contact sensitisation, despite the responder/non-responder donor effect they observed.

Aiba *et al.* (47), reported significant up-regulation of CD86, CD54 and HLA-DR antigen and an increase in the IL-1β protein production/secretion in response to the sensitiser, dinitrochlorobenzene (DNCB), and nickel chloride ($NiCl_2$) in a Mo-DC model derived in the presence of GM-CSF and IL-4. In addition, $NiCl_2$ exposure also increased IL-6 and TNF-α production. A significant up-regulation of HLA-DR and CD86 was also observed following exposure to DNCB. Aiba *et al.* (48) evaluated the response of TGF-β1-treated MoDCs to $NiCl_2$ and DNCB. Several markers related to maturation and migration processes of LCs were found to be modulated by $NiCl_2$ exposure. The effects of DNCB were less pronounced, but nevertheless, CD86 membrane marker and CCR7 mRNA were up-regulated, and E-cadherin and CLA were down-regulated. The response of Mo-DCs exposed to several organic contact sensitisers or to metals has also been studied (44–51). As previously observed, DC cultures mostly responded to challenge by the regulated expression of membrane determinants, such as CD86 or HLA-DR, or the production of TNF-α. Most importantly, skin irritants and DNCB did not have any effect on surface marker expression in any these studies.

Recently, results obtained on the typical phenotypic changes of DC-maturation (altered expression of HLA-DR, CD1a, CD40, CD54, CD83, CD86, CCR7 and E-cadherin) have suggested that a 4-day treatment of Mo-DCs with chemical allergens resulted in the most robust phenotypic changes (52). A high variability in the response of DCs according to their donors was also observed.

Aeby *et al.* (53) evaluated CD86 surface marker expression and IL-1β and aquaporin 3 mRNA expression in Mo-DCs, pooled from four different donors and challenged with 2,4,6-trinitrobenzenesulphonic acid (TNBS), two related aromatic amines, p-toluylenediamine (PTD) and hydroxyethyl-p-phenylenediamine (HE-PPD), and SDS for up to 30 hours. TNBS was shown to induce a dose-dependent response for all the parameters tested, whereas SDS did not. Also, both PTD and HE-PPD induced DC-activation. However, HE-PPD is not classified as a sensitiser by the *in vivo* LLNA assay. Skin penetration studies indicated that the differences among *in vitro* and *in vivo* studies were due to bioavailability differences. The authors suggest that, in order to establish a robust prediction model, additional correction factors for bioavailability, *in vitro* metabolism and *in vitro* protein binding have to be addressed.

Modest increases in IL-1β mRNA expression in the response of Mo-DCs to several contact allergens, but not to irritants, were described by Reutter *et al.* (26) and Pichowski *et al.* (27, 28) when using short exposure periods. These results suggest that this marker is probably of limited predictive value under such conditions. It is possible, however, that IL-1β may prove to be of greater value in longer-exposure protocols.

CD34-DCs

An advantage of using CD34-DCs is that substantially higher yields of cells are obtained than with Mo-DC cultures, thereby allowing more chemicals to be tested with cells from one donor (29).

Rougier *et al.* (54, 55) exposed CD34-DCs to strong haptens, weak sensitisers and one irritant. The main endpoint investigated was T-cell activation; however, CD86, CD83, CD1a, E-cadherin and HLA-DR expression were also measured for the weak sensitisers. Based on the two studies, it can be concluded that a 48-hour contact of immature (8-day cultured) CD34-DCs with strong haptens triggers phenotypic (high CD86, CD83 and HLA-DR) and functional (T-cell activation) maturation.

De Smedt *et al.* (56, 57) have shown that the CD34-DC system has the capacity to distinguish between sensitisers and irritants, but also that the analysis of several surface markers was necessary. The phenotypic response was more sensitive and specific in comparison with cytokine production. However, not all the test chemicals were able to provoke a response with respect to all the surface markers analysed. Exposure concentration was also an important factor. There was no correlation between relative potency of the sensitiser and any of the endpoints analysed (57).

De Smedt *et al.* (29) compared the performance of CD34-DCs and Mo-DCs in *in vitro* models for detecting contact allergens. Mo-DCs and CD34-DCs were exposed to several contact allergens and one skin irritant. The endpoints analysed were the membrane expression of CD86, CD83 and HLA-DR, and IL-1β production. Both test systems were able to respond to allergens, but CD34-DCs had to be exposed to higher concentrations to demonstrate significant phenotypic changes. With Mo-DCs, only a fraction of donors yielded cells that were responsive to allergens, which is in contrast to the experience with CD34-DCs, where all the donors

responded in a similar fashion. CD86 was the most reliable marker for the precise separation of sensitisers and non-sensitisers in both culture systems. Finally, CCR7 up-regulation on CD34-DCs has been described, following exposure to nickel and DNCB (58).

Microarray analysis

From the review presented above, it is clear that the endpoints that are analysed are mainly known membrane markers, such as CD86 and HLA-DR, and cytokine expression and/or production. More-recent studies have included additional markers, such as CCR7 and aquaporin 3 (53, 58, 59). However, several studies indicate that the response patterns of the markers currently used may be chemical-dependent, do not allow the identification of all sensitisers, and may have a limited dynamic range. In addition, the use of Mo-DCs involves the problem of the responder/non-responder donor phenotype. Therefore, multiple endpoint measurements may be necessary to identify chemical allergens, but, even under these circumstances, no validated methods are currently available.

One important initiative has been to explore more holistically, changes in gene expression that may be associated in a selective fashion with exposure of cultured DCs to contact allergens. For this purpose, there has been considerable interest in the use of microarray platforms for multiple transcript profiling.

Several microarray studies have been published, in which Mo-DCs have been exposed to known maturation stimuli, such as bacterial lipopolysaccharide (LPS) or CD40 ligand (60–66). These studies have begun to provide information about the changes in gene expression which occur during induced DC maturation processes, and have highlighted some differences between stimuli. These studies may result in the identification of markers appropriate for measuring allergen-induced changes in DC maturation.

Recently, Ryan *et al.* (67) described the transcriptional profile of Mo-DCs exposed to the chemical allergen, dinitrobenzenesulphonic acid (DNBS). Several genes were identified that should be investigated further for their capacity to discriminate between contact sensitisers and non-allergens.

Signal transduction in DCs

As already mentioned above, contact sensitisers are able to induce DC maturation *in vitro*. Other markers of DC function have been shown to be up-regulated by haptens in a manner similar to that observed with LPS, pro-inflammatory cytokines or CD40 ligand. The question remains as to whether haptens use signal tranduction pathways in common with other types of danger signals to activate DCs, or whether they use other pathways. An associated question is whether haptens can be classified on the basis of their signal transduction profiles.

Tyrosine phosphorylation has been observed as a common cellular response to contact allergens in a variety of MHC class II-positive cells, including LCs and Mo-DCs (68). The induction of p-tyr by strong haptens was noted in all cases, indicating the importance of signal-transduction mechanisms in the response to contact allergens.

Several transduction pathways have been described to play a role in DC maturation and survival, of which the mitogen-activated protein kinases (MAPKs), NFκB and cAMP pathways are the most fully-characterised.

MAPKs are composed of three families, namely, extracellular regulated kinases (ERKs), jun kinases (JNKs) and p38 MAPK. The JNK and p38 MAPK cascades are referred as stress-activated MAPK pathways, because they are strongly activated by stress-inducing agonists such as LPS, inflammatory cytokines or xenobiotics. MAPKs play a major role in DC maturation induced by LPS through Toll-like receptors (69, 70), or by inflammatory cytokines through binding to specific receptors (71). Haptens. such as nickel, DNCB and DNFB, induce a rapid phosphorylation of p38 MAPK and JNKs in both Mo-DCs differentiated in the presence of TGF-β, and in CD34-DCs (72, 58, 59). Recently, ERKs have also been shown to be phosphorylated in response to $NiSO_4$ or DNCB in CD34-DCs and by $NiCl_2$ in Mo-DCs (58, 59, 72). It is important to appreciate that responses may differ according to the DC model used. Indeed, with Mo-DCs differentiated in the absence of TGF-β JNKs were not activated by $NiSO_4$, and only a slight increase in ERK phosphorylation was observed, suggesting that nickel may mobilise MAPKs differently, depending on the origin of the human DCs (73).

The availability of reagents such as aminomethoxyflavone (PD98059), pyridinyl imidazole (SB203580) and anthrapyrazolone (SP600125), which inhibit ERKs, p38 MAPK, and JNKs respectively, has allowed great progress to be made in this field, although they are not totally specific for the targets for which they were designed (74). The inhibition of p38 MAPK correlates with alteration in phenotypic markers of maturation, such as CD86, HLA-DR or CD83 in Mo-DCs differentiated in the presence of TGF-β and stimulated with nickel or DNCB (72). However, the inhibition of p38 MAPK before the addition of nickel to Mo-DCs differentiated in the absence of TGF-β affected both HLA-DR and CD83 expression, but did not significantly modulate CD86 expression (73). With CD34-DCs, inhibition of p38 MAPK was also correlated with alterations in CD86, CD83 and CCR7 expression upon the addition of $NiSO_4$ or DNCB (59).

The role of JNKs has only been addressed in CD34-DCs, where the results indicated that inhibition of JNKs by SP600125 down-regulated CD86, CCR7 and CD83 in response to nickel (59). Similar results have been reported by the same group for ERKs in the same model when using $NiSO_4$ stimulation, in contrast to what has been described after the addition of LPS (69, 73).

Interestingly, the inhibition of the MAPKs by pharmacological inhibitors did not affect the down-regulation of langerin and E-cadherin by $NiSO_4$, suggesting that these pathways are not involved (59).

Inhibitors of the NFκB pathway are also available. However, they show some cytotoxicity in DC models, and there are no reports of the effect of NFκB inhibition following hapten addition. A recent report, in which small interfering RNA (siRNA) for p50NFκB was used, has shown that this pathway plays a major role in IL-12p40 gene expression (75). Nevertheless, it has been shown that $NiCl_2$ treatment can lead to the activation of NFκB, suggesting that this pathway is probably involved in DC activation by some haptens (72).

Several groups have recently shown, by using Mo-DCs cultured in the presence or absence of serum, that signals provided by prostaglandin E2 (PGE2) were required for the migration of mature DCs in response to CCL19 and CCL21 (76, 77). However, the efficient migration of CD34-DCs was stimulated by haptens without the addition of lipid mediators, suggesting that AMPc production is not a prerequisite for DC migration in this model (58). These results again emphasise differences between the Mo-DC and CD34-DC models.

Models assessing DC–T lymphocyte interactions

LCs present haptens to T lymphocytes in the paracortical regions of lymph nodes. Hapten-specific T CD4+ and CD8+ lymphocytes are then activated, resulting in an expansion of hapten-specific T lymphocytes. For many years, the contact hypersensitivity reaction was considered to be a cutaneous inflammatory reaction mediated by T CD4+ lymphocytes. More recently, evidence has emerged for the important participation of T CD8+ lymphocytes in contact hypersensitivity (78–80), in both mice and humans (10, 81). Hauser & Katz (82) reported the capacity of cultured LCs to induce the priming of hapten-specific naïve T lymphocytes. These results have been confirmed by others (83, 84), by using LCs isolated from the epidermis or from blood lymphocytes. Indeed, by culturing T lymphocytes and LCs for 2 days with trinitrophenyl (TNP), lymphocyte proliferation could be measured. Epidermal LCs, cultured for 48 hours, mature spontaneously and may thus represent LCs that have migrated to regional lymph nodes (83, 85, 86).

The functional potency of CD34-DCs was evaluated by the mixed lymphocyte reaction (MLR; 87, 88) or antigenic presentation to antigen-specific T-cell clones (37, 88) or hapten presentation to naïve T-cells (54, 55, 89–91).

Mature DCs that have been cultured for 10–14 days and that activate T lymphocytes, correspond to a spontaneous maturation of DCs, as suggested by the emergence of markers of co-stimulation, such as CD86, CD80, CD54 and CD58, and the marker of maturation, CD83 (54). With these spontaneously matured DCs, only strong haptens can activate leukocytes. Indeed, a positive proliferative response was obtained with TNBS, fluorescein isothiocyanate (FITC) and Bandrowski's base (BB) in all the experiments, whereas negative results were obtained with coumarin and citronellal, in agreement with previous results reported by Krasteva *et al.* (84) and those found in human predictive skin testing (92). On the other hand, the use of immature DCs and 48 hours of culture prior to contact with lymphocytes, allows the activation of lymphocytes by strong haptens and by weak sensitisers in some cultures. A frequency of positive responses with paraphenylenediamine (PPD), citronellal and coumarin has been reported by Krasteva *et al.* (84), and may be related to the low frequency of positive reactions with fragrance material and weak sensitisers *in vivo* (93–96). PPD is a strong sensitiser in all animal and human *in vivo* predictive tests (97–100). The molecule is not itself active, but is considered to be a prohapten that is converted metabolically to an active sensitiser. To date, no *in vitro* tests have succeeded in detecting a direct effect of this sensitiser, whereas BB, one of its oxidation products, regularly induces a primary sensitisation (54, 84). Consequently, the absence of T-cell proliferation with PPD in most of the experiments may be due to the low metabolic competence of DCs.

Integration of DCs into organotypic models

While cultured DCs or cell lines are promising tools for identifying skin sensitisation hazards, risk assessment requires information on potency and therefore more-sophisticated tools, if the aim is a complete elimination of the need for experimental animals.

Mimicking the target organ, the viable epidermis in this case, with organotypic models that would include functional DCs, appears to be a logical and attractive strategy for addressing this problem. Organotypic models can display a barrier function and metabolic properties that, in theory, would permit consideration of the roles of these factors in the acquisition of sensitisation. Reconstructed skin

would also enable the development of an experimental system that would facilitate a more-relevant exposure regimen.

The first successful integration of human epidermal LC-like cells derived from CD34+ haematopoietic progenitor cells into a reconstructed human epidermis, was reported on de-epidermised epidermis in 1997 (101). Eventually, LC-like cells derived from CD34+ cells were also successfully introduced into other epithelial models, such as the oral mucosa (102) or the vaginal mucosa (103). However, there are as yet no data available on the use of such models for examining the changes induced by chemical sensitisers.

A second-generation human epidermis model containing functional LC-like cells was recently described (104). This model was engineered on a collagen dermal equivalent made of cross-linked collagen I surfaced with collagen IV, and can be produced at an industrial level. Within this epidermis, CD34+-derived LC-like cells are located in the suprabasal cell layers at a density comparable to that of normal human skin (105). Importantly, whereas LCs normally emigrate spontaneously from the epidermis (106), these cells were found to remain resident within the period of culture. This human reconstructed epidermis exhibits a well-stratified epidermis with LC-like cells located in its suprabasal layers. Immunostaining of the LC in the respective epidermal sheet revealed a dense network of equally distributed cells. As in the initial model, the integrated LCs exhibited all the characteristic features of epidermal LC, such as Birbeck granules and CDla surface antigens (101).

Exposure of the reconstructed skin to known sensitisers (DNFB, $NiSO_4$, benzocaïne, oxazolone, eugenol), or to SDS, affected the integrated LC-like cells in ways similar to those described for their *in vivo* counterparts. Topically applied sensitisers induced a reduction in the number of cells and affected their characteristic dendritic morphology (fewer and shorter dendrites), whereas the irritant (SDS) induced no changes, confirming the early observation made by Aiba *et al.* (47), that DCs respond differently to haptens and irritants. The same holds true for the up-regulation of the surface antigen CD86 observed in the reconstructed epidermis after exposure to haptens (48). It was shown that the up-regulation of CD86 was specific for the integrated LCs, since keratinocytes did not express CD86 under these experimental conditions.

Human myeloid cell lines

Due to the inherent variability among human donors and the difficulty in standardising the protocol for generating DC-like cells, the need for a more homogeneous and reproducible material was rapidly identified, with special attention to the need for cell lines. The first results were obtained with the murine XS52 DC cell line (107), demonstrating that cell lines can be used to discriminate between contact sensitisers and non-sensitisers. Due to unavailability of human DC lines, various groups have postulated that myeloid human cell lines, which belong to the same broad haematopoietic lineage as DC, and which are known to differentiate in response to various stimuli, may have the capacity to respond to chemical allergens. The abilities of a variety of human myeloid cell lines, from the very immature KG-1, to the pro-monocytic U937 and THP-1 cell lines, to differentiate toward a DC phenotype and to respond to contact sensitisers, have been investigated.

In response to cytokine cocktails, the CD34+ myelomonocytic cell line, KG-1, can be induced to differentiate into DC-like cells, as demonstrated by phenotypic, morphological and functional changes (51, 108). However, differentiated KG-1 cells did not provide a sensitive *in vitro* method for assessing the sensitisation potential of chemicals.

Parallel investigations were conducted with two pro-monocytic cell lines, U937 and the slightly more mature THP-1. Both cell lines differentiated in response to stimuli such as cytokines (GM-CSF, IL-4, TNF-α) or phorbolmyristate acetate (PMA), and acquired some DC characteristics. The appearance of typical DC surface markers, such as CD1a, CD83 and high CD86 expression, was observed (109–112).

As indicated by the up-regulation of the co-stimulatory surface molecule, CD86, and the adhesion molecule, CD54, as well as the increased internalisation of MHC-class II molecules (109, 110), both these cell lines appear to respond selectively to contact sensitisers. For the THP-1 cell line, these effects were observed after 24-hour incubation, but only with undifferentiated cells (109, 110).

These results suggest that, under adequate culture and exposure conditions, the undifferentiated U-937 or THP-1 cell lines may provide useful tools for examining the interactions of chemicals with DC-like cells. The *in vitro* identification of contact allergens through the modulation of human myeloid cell lines warrants further investigation.

General Considerations

As with any other toxicological assay, consideration must be given to the compatibility of the test agent with the *in vitro* test system. For example, with the LLNA, the open topical application of materials to the ears means that entirely aqueous vehicle systems are likely to generate sub-optimal results and so should be avoided (113, 114). In contrast, with cell culture systems, particularly those based on suspension culture, as is the case with DC cultures, there may be practical challenges associated with the assessment of very hydrophobic substances;

such materials are associated with skin sensitisation.

Also of importance is that *in vitro* methods for skin sensitisation are, in practice, most likely to form part of an integrated, tiered safety assessment strategy. For example, a positive result from a DC-based assay may need to be considered in the context of pharmacokinetic data, in order for a safety assessment to be undertaken, as well as in association with structural data. Where there are multiple data, an additional challenge will be how to weight the varying sources of evidence in situations where elements of that evidence are contradictory.

Interspecies differences

Evidence from *in vivo* tests indicates that interspecies differences (guinea-pig *versus* mouse *versus* human) are relatively limited (115), as indeed are differences in potency (116, 117). Differences do occur, however. It is reasonable to assume that species-specific differences in skin metabolism may have an impact on the predictive identification of sensitisation hazards. However, care should be taken not to assume that current knowledge can be applied directly to the cellular level of exposure associated with *in vitro* test models. A particular point of difference may well arise at the level of metabolism, which is an area where knowledge is limited (118, 119). It is probable that single cell test systems will, at best, imperfectly represent the full range of metabolic opportunities available *in vivo*.

Comparison with *in vivo* data

Great care must be taken in the selection of data against which to assess an *in vitro* method, and also in choosing the criteria for distinguishing between sensitisers and non-sensitisers. The approaches adopted in the validation of the LLNA and the evaluation of the data produced are recommended (116). The further publication of LLNA data and comparison with human data should be encouraged, to expand the number of chemicals and the chemical range of the dataset, in such a way that it is available prior to any potential formal validation activity. Human data on the presence of skin sensitisation hazard should normally represent the gold standard, with the intent of the validation being that an *in vitro* test or testing strategy should predict the human perspective as accurately as does the LLNA; this, of course, applies to the potential potency of an identified sensitiser, not to the risk, which will also depend on exposure variables. Where a discrepancy exists, priority should be given to human data (120, 121).

The availability of models

Any model being entered into (pre)validation must be freely available, whether or not it is protected (for example, by a patent); this includes the free availability of a highly detailed protocol, such that any suitably qualified laboratory has an opportunity to accurately reproduce the model in its entirety. Data generated in validation-related studies should not form part of further patenting.

Nickel and other metals

It is recognised that the sensitising potential of metals is already well characterised (122), so they should not form a significant part of any reference chemical dataset to be used in the development or validation of *in vitro* alternatives for predicting skin sensitisation. Furthermore, nickel is commonly used as a model skin sensitiser in investigative mechanistic studies. Information derived from such work should be interpreted with a degree of caution in terms of the development of *in vitro* assays, since nickel represents an unusual class of sensitiser and is unrepresentative of the large majority of organic sensitisers. Organic chemical sensitisers form hapten-protein complexes by covalent bond formation; in contrast, nickel binds via the formation of coordination complexes (118, 123, 124).

Bioactives

The evaluation of bioactives may be problematical with *in vivo* models; for example, they may interfere with the normal immunobiological processes involved in the induction of skin sensitisation. Steroids represent a good example of this phenomenon; hydrocortisone and tixocortol pivalate are negative in predictive *in vivo* testing (David Basketter, personal communication), no doubt because they tend to inhibit key pathways associated with the induction of sensitisation. Similar issues should be anticipated for *in vitro* models.

Quantitative risk assessment

Existing *in vivo* tests for skin sensitisation provide not only hazard identification, but also the information on allergen potency which is required for risk assessment (125, 126). It is important that *in vitro* methods are ultimately able to provide a similar quality of information, so that the accuracy of human safety evaluations is not compromised. To achieve this goal, it may ultimately prove necessary to derive a sophisticated view on the integration of related elements that combine to determine the intrinsic potency of a chemical allergen. Currently,

the immunological events associated with potency are poorly understood.

Contact *versus* respiratory sensitisers

The current *in vivo* assays detect both classes of sensitiser, largely because chemical respiratory sensitisers are normally also capable of causing contact sensitisation (127). So far, however, this has not caused any difficulties of interpretation, probably as a consequence of the overwhelming preponderance of contact sensitisers. However, consideration should be given as to whether, and in what manner, this may be true of mechanistically based *in vitro* assays.

General Observations and Recommendations

1. It is now possible to maintain human DCs of various phenotypes in short-term culture. Such cells provide a suitable approach for the development of *in vitro* methods appropriate for the characterisation of human sensitisation hazard.

2. It is important to recognise that methods based on DCs alone may not provide a complete answer; it may be necessary to develop integrated approaches that incorporate more than one (i.e. complementary) method.

3. *In vitro* methods suitable for routine use for skin sensitisation testing should be relevant for a wide range of chemical classes.

4. In common with other *in vitro* approaches, it is important to consider the requirement for metabolic competence. It is estimated that a significant number of contact allergens are prohaptens and require metabolic activation for the induction of sensitisation.

5. It is necessary to develop appropriate evaluation and validation strategies. An approach similar to that used for the LLNA is recommended.

6. To facilitate evaluation and validation, it is necessary to have available chemicals that are of known sensitising potential (and of different potencies), and materials that are known not to induce skin sensitisation. The European Centre for the Ecotoxicology and Toxicology of Chemicals (ECETOC) database is appropriate for this purpose.

7. It is also necessary to investigate carefully the ability of prospective test methods to distinguish between contact allergens and skin irritants.

8. An ideal method for skin sensitisation testing should not only identify hazard, but should also provide information on likely sensitisation potency (hazard characterisation). In this context, it is recommended that any proposed methods should be investigated for their ability to distinguish between chemical allergens of varying sensitising potencies.

9. The LLNA is able to identify chemical allergens that are, in humans, associated primarily with sensitisation in the respiratory tract. It is recommended that new test methods are investigated for their ability to identify chemical allergens of different classes.

10. Ideally, novel test methods should be mechanistically based on an understanding of the relevant biological events that are associated with, and/or required for, the acquisition of skin sensitisation.

11. If novel tests are based on the use of primary cell cultures, consideration needs to be given to the possibility of cryopreservation. This, in turn, will require an understanding of the extent to which freezing cells under various conditions affects their biological properties.

12. *In vitro* tests rely on assessment of changes induced by exposure to test chemicals. It is necessary to develop a strategy with regard to the relevance of cell injury or cellular cytotoxicity. Associated with this is a requirement to determine the best and most appropriate methods for measuring cell viability, before, during and/or after exposure to test materials.

13. Particularly in the context of DC-based methods, there is a need to establish definitively what level of cellular differentiation is required for optimal test performance, and how best to ensure that this is achieved within acceptable tolerance limits.

14. With respect to assays based on the use of continuous cell lines, there is a need to ensure uniformity between laboratories in terms of culture conditions and acceptable phenotypic characteristics.

15. Ideally, the continuous cell lines used in skin sensitisation testing should be readily available from commercial sources, and should be robust and easy to culture.

16. For all methods, prior to formal validation, details should be available of which parameters

of test performance are acceptable, either for use in a stand-alone method for hazard identification, or for use in a screening test.

Future Research

17. There is a need to understand the ways in which chemicals interact with cells in culture to provoke changes in phenotype and/or function which are reflective of skin sensitising activity.

18. There is a need for more-standardised and more-refined methods for the isolation and characterisation of DCs derived from precursors in human peripheral blood.

19. There is available evidence for functional heterogeneity between DCs derived from different donors. There is a need to understand the mechanistic bases of such variations.

20. There is a need to consider, within the context of each cellular read-out system, the most important and most relevant phenotypic and functional changes that will serve as endpoints for skin sensitisation potential.

21. It is known that factors other than chemical allergens (such as proteins and lipopolysaccharides) are able to impact on the phenotype and function of cultured DCs and continuous cell lines. There is a need to understand, in greater detail, whether and to what extent such factors may impact on the integrity of cellular methods for skin sensitisation hazard.

22. A more-detailed appreciation is required of the influence of cellular viability on the performance of cell-based assays for skin sensitisation. For instance, it is not yet clear, in each of the proposed systems, whether any loss of cellular viability should be avoided, or whether a certain level of cellular trauma is required for full responses to contact allergens (i.e. the need for danger signals).

23. There is a need for more-detailed understanding of the extent to which cultured DCs and continuous cell lines possess metabolic activity, and whether this is sufficient for the routine identification of pro-haptens.

24. If endogenous metabolic activity is absent or insufficient for the transformation of pro-haptens, consideration must be given to the most appropriate sources of exogenous metabolic function

Conclusions

Exciting opportunities are now available for exploring the potential of cultured DCs, or of continuous cell lines of haematopoeitic origin, to provide the basis for *in vitro* for skin sensitisation testing. Highlighted here are recommendations for the further development, and subsequent evaluation and validation, of such methods, and requirements for further research.

References

1. Buehler, E.V. (1965). Delayed contact hypersensitivity in the guinea pig. *Archives of Dermatology* **91**, 171–177.
2. Magnusson, B. & Kligman, A.M. (1970). *Allergic Contact Dermatitis in the Guinea Pig: Identification of Contact Allergens*, 141pp. Springfield, IL, USA: Charles C Thomas.
3. Kimber, I., Dearman, R.J., Basketter, D.A., Ryan, C.A. & Gerberick, G.F. (2002). The local lymph node assay: past, present and future. *Contact Dermatitis* **47**, 315–328.
4. Kimber, I., Pichowski, J.S., Basketter, D.A. & Dearman, R.J. (1999). Immune responses to contact allergens: novel approaches to hazard evaluation. *Toxicology Letters* **106**, 237–246.
5. Kimber, I., Pichowski, J.S., Betts, C.J., Cumberbatch, M., Basketter, D.A. & Dearman, R.J. (2001). Alternative approaches to the identification and characterization of chemical allergens. *Toxicology in Vitro* **15**, 307–312.
6. Ryan, C.A., Hulette, B.C. & Gerberick, G.F. (2001). Approaches for the development of cell-based *in vitro* methods for contact sensitisation. *Toxicology in Vitro* **15**, 43–55.
7. Kimber, I., Cumberbatch, M., Betts C.J. & Dearman, R.J. (2004). Dendritic cells and skin sensitisation hazard assessment. *Toxicology in Vitro* **18**, 195–202.
8. Grabbe, S. & Schwarz, T. (1998). Immunoregulatory mechanisms involved in the elicitation of allergic contact hypersensitivity. *Immunology Today* **19**, 37–44.
9. Kimber, I., Basketter, D.A., Gerberick, G.F. & Dearman, R.J. (2002). Allergic contact dermatitis. *International Immunopharmacology* **2**, 201–211.
10. Kimber, I. & Dearman, R.J. (2002). Allergic contact dermatitis: the cellular effectors. *Contact Dermatitis* **46**, 1–5.
11. Sebastiani, S., Albanesi C., De Pita, O., Puddu, P., Cavani, A. & Girolomoni, G. (2002). The role of chemokines in allergic contact dermatitis. *Archives of Dermatological Research* **293**, 552–559.
12. Dearman, R.J. & Kimber, I. (2003). Factors influencing the induction phase of skin sensitisation. *Americal Journal of Contact Dermatitis* **14**, 188–194.
13. Friedmann, P.S. (1981). The immunobiology of Langerhans cells. *Immunology Today* **2**, 124–128.
14. Kimber, I. & Cumberbatch, M. (1992). Dendritic cells and cutaneous immune responses to chemical allergens. *Toxicology and Applied Pharmacology* **117**, 137–146.
15. Kimber, I., Dearman, R.J., Cumberbatch, M. & Huby, R.J.D. (1998). Langerhans cells and chemical allergy. *Current Opinion in Immunology* **10**, 614–619.

16. Kimber, I., Cumberbatch, M., Dearman, R.J., Bhushan, M. & Griffiths, C.E.M. (2000). Cytokines and chemokines in the initiation and regulation of epidermal Langerhans cell mobilization. *British Journal of Dermatology* **142**, 401–412.
17. Maurer, D. & Stingl, G. (2001). Lagerhans cells. In *Dendritic Cells: Biology and Clinical Applications* (ed. M T. Lotze & A.W. Thomson), 2nd edn, pp. 35–50. San Diego, CA, USA: Academic Press.
18. Cumberbatch, M., Dearman, R.J., Griffiths, C.E.M. & Kimber, I. (2000). Langerhans cell migration. *Clinical and Experimental Dermatology* **25**, 413–418.
19. Cumberbatch, M., Dearman, R.J., Griffiths, C.E.M. & Kimber, I. (2003). Epidermal Langerhans cell migration and sensitisation to chemical allergens. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* **111**, 797–804.
20. Kimber, I., Cumberbatch, M., Dearman, R.J. & Griffiths, C.E.M. (2002). Danger signals and skin sensitisation. *British Journal of Dermatology* **147**, 613–614.
21. Enk, A.H. & Katz, S.I. (1992) Early molecular events in the induction phase of contact sensitivity. *Proceedings of the National Academy of Sciences USA* **89**, 1398–1402.
22. de Silva, O., Basketter, D.A., Barratt, M.D., Corsini, E., Cronin, M.T.D., Das, P.K., Degwert, J., Enk, A., Garrigue, J.L., Hauser, C., Kimber, I., Lepoittevin, J-P., Peguet, J. & Ponec, M. (1996). Alternative methods for skin sensitisation testing. The report and recommendations of ECVAM workshop 19. *ATLA* **24**, 683–705.
23. Enk, A.H., Angeloni, V.L., Udey, M.C. & Katz, S.I. (1993). An essential role of Langerhans cell-derived IL-1β in the initiation of primary immune responses in skin. *Journal of Immunology* **150**, 3698–3704.
24. Shornick, L.P., De Togni, P., Mariathasan, S., Goellner, J., Strauss-Schoenberger, J., Karr, R.W., Ferguson, T.A. & Chaplin, D.D. (1996). Mice deficient in IL-1β manifest impaired contact hypersensitivity to trinitrochlorobenzene. *Journal of Experimental Medicine* **183**, 1427–1436.
25. Cumberbatch, M., Dearman, R.J. & Kimber, I. (1997). Langerhans cells require signals from both tumour necrosis factor-α and interleukin-1β for migration. *Immunology* **92**, 388–395.
26. Reutter, K., Jager, D., Degwert, J., & Hoppe, U. (1997). *In vitro* model for contact sensitisation. II. Induction of IL-1β mRNA in human blood-derived dendritic cells by contact sensitisers. *Toxicology in Vitro* **11**, 619–626.
27. Pichowski, J.S., Cumberbatch, M., Dearman, R.J., Basketter, D.A. & Kimber, I. (2000). Investigation of induced changes in interleukin 1β mRNA expression by cultured human dendritic cells as an *in vitro* approach to skin sensitisation testing. *Toxicology in Vitro* **14**, 351–360.
28. Pichowski, J.S., Cumberbatch, M., Dearman, R.J., Basketter, D.A. & Kimber, I. (2001). Allergen-induced changes in interleukin 1 beta (IL-1 beta) mRNA expression by human blood-derived dendritic cells: inter-individual differences and relevance for sensitisation testing. *Journal of Applied Toxicology* **21**, 115–121.
29. De Smedt, A.C.A., Van Den Heuvel, R.L., Van Tendeloo, V.F.I., Berneman, Z.N., Schoeters, G.E.R., Weber, E. & Tuschl, H. (2002). Phenotypic alterations and IL-1β production in CD34+ progenitor- and monocyte-derived dendritic cells after exposure to allergens: a comparative analysis. *Archives of Dermatological Research* **294**, 109–116.
30. Strunk, D., Rappersberger, K., Egger, C., Strobl, H., Krömer, E., Elbe, A., Maurer, D. & Stingl, G. (1996). Generation of human dendritic cells/Langerhans cells from circulating CD34+ hematopoietic progenitor cells. *Blood* **87**, 1292–1302.
31. Santiago-Schwarz, F., Belios, E., Diamond, B. & Carson, S.E. (1992). TNF in combination with GM-CSF enhances the differentiation of neonatal cord blood cells into dendritic cells and macrophages. *Journal of Leucocyte Biology* **52**, 274–281.
32. Caux, C., Vanbervliet, B., Massacrier, C., Dezutter-Dambuyant, C., de Saint-Vis, B., Jacquet, C., Yoneda, K., Imamura, S., Schmitt, D. & Banchereau, J. (1996). CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF-α. *Journal of Experimental Medicine* **184**, 695–706.
33. Arrighi, J.F., Hauser, C., Chapuis, B., Zubler, R. & Kindler, V. (1999). Long-term culture of human CD34+ progenitors from FLT3-ligand, thrombopoietin, and stem cell factor induces extensive amplification of a CD34-CD14- and a CD34-CD14+ dendritic cell precursor. *Blood* **93**, 2244–2252.
34. Curti, A., Fogli, M., Ratta, M., Tura, S. & Lemoli, R.M. (2001). Stem cell factor and FLT3-ligand are strictly required to sustain the long-term expansion of primitive CD34+DR- dendritic cell precursors. *Journal of Immunology* **166**, 848–854.
35. Jaksits, S., Kriehuber, E., Charbonnier, A.S., Rappersberger, K., Stingl, G. & Maurer, D. (1999). CD34+ cell-derived CD14+ precursor cells develop into Langerhans cells in a TGF-β1-dependent manner. *Journal of Immunology* **163**, 4869–4877.
36. Ferlazzo, G., Wesa, A., Wei, W.Z. & Galy, A. (1999). Dendritic cells generated from CD34+ progenitor cells or from monocytes differ in their ability to activate antigen-specific CD8+ T cells. *Journal of Immunology* **163**, 3597–3604.
37. Sallusto, F. & Lanzavecchia, A. (1994). Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony stimulating factor plus interleukin-4 and down-regulated by tumor necrosis factor alpha. *Journal of Experimental Medicine* **179**, 1109–1118.
38. Sallusto, F., Cella, M., Danieli, C. & Lanzavecchia, A. (1995). Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: down-regulation by cytokines and bacterial products. *Journal of Experimental Medicine* **182**, 389–400.
39. Snijders, A., Kalinski, P., Hilkens, C.M & Kapsenberg, M.L. (1998). High-level IL-12 production by human dendritic cells requires two signals. *International Immunology* **10**, 1593–1598.
40. Santini S.M., Lapenta, C., Logozzi, M., Parlato, S., Spada, M., Di Pucchio, T. & Belardelli, F. (2000). Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity *in vitro* and in Hu-PBL-SCID mice. *Journal of Experimental Medicine* **15**, 1777–1788.
41. Geissmann, F., Prost, C., Monnet, J.P., Dy ,M., Brousse, N. & Hermine, O. (1998). Transforming growth factor beta 1, in the presence of granulocyte/macrophage colony-stimulating factor and interleukin 4, induces differentiation of human

peripheral blood monocytes into dendritic Langerhans cells. *Journal of Experimental Medicine* **16**, 961–966.

42. Verrier, A.C., Schmitt, D. & Staquette, M-J. (1999). Fragrance and contact allergens *in vitro* modulate the HLA-DR and E-cadherin expression on human epidermal Langerhans cells. *International Archives of Allergy and Immunology* **120**, 56–62.
43. Rizova, H., Carayon, P., Barbier, A., Lacheretz, F., Dubertret, L. & Michel, L. (1999). Contact allergens, but not irritants, alter receptor-mediated endocytosis by human epidermal Langerhans cells. *British Journal of Dermatology* **140**, 200–209.
44. Tuschl, H. & Kovac, R. (2001). Langerhans cells and immature dendritic cells as model systems for screening of skin sensitisers. *Toxicology in Vitro* **15**, 327–331.
45. Degwert, J., Steckel, F., Hoppe, U. & Kligman, L.H. (1997). *In vitro* model for contact sensitisation. I. Stimulatory capacities of human blood-derived dendritic cells and their phenotypical alterations in the presence of contact sensitisers. *Toxicology in Vitro* **11**, 613–618.
46. Becker, D., Kühn, U., Lempertz, U., Enk, A., Saloga, J. & Knop, J. (1997). Flow-cytometric screening for the modulation of receptor-mediated endocytosis in human dendritic cells: implications for the development of an *in vitro* technique for predictive testing of contact sensitisers. *Journal of Immunological Methods* **203**, 171–180.
47. Aiba, S., Terunuma, A., Manome, H. & Tagami, H. (1997). Dendritic cells differently respond to haptens and irritants by their production of cytokines and expression of co-stimulatory molecules. *European Journal of Immunology* **27**, 3031–3038.
48. Aiba, S., Manome, H., Yoshino, Y. & Tagami, H. (2000). *In vitro* treatment of human transforming growth factor-1β-treated monocyte-derived dendritic cells with haptens can induce the phenotypic and functional changes similar to epidermal Langerhans cells in the initiation phase of allergic contact sensitivity reaction. *Immunology* **101**, 68–75.
49. Coutant, K.D., de Brugerolle de Fraissinette, A., Cordier, A. & Ulrich, P. (1999). Modulation of the activity of human monocyte-derived dendritic cells by chemical haptens, a metal alleren, and a Staphylococcal superantigen. *Toxicological Sciences* **52**, 189–198.
50. Manome, H., Aiba, S. & Tagami, H. (1999). Simple chemicals can induce maturation and apoptosis of dendritic cells. *Immunology* **98**, 481–490.
51. Hulette, B.C., Ryan, C.A. & Gerberick, G.F. (2002). Elucidating changes in surface marker expression of dendritic cells following chemical allergen treatment. *Toxicology and Applied Pharmacology* **182**, 226–233.
52. Staquet, M.J., Sportouch, M., Jacquet, C., Schmitt, D., Guesnet, J. & Péguet-Navarro, J. (2004). Moderate skin sensitisers can induce phenotypic changes on *in vitro* generated dendritic cells. *Toxicology in Vitro* **18**, 493–500.
53. Aeby, P., Wyss, C., Beck, H., Griem, P., Scheffler, H. & Goebel, C. (2004). Characterization of the sensitizing potential of chemicals by *in vitro* analysis of dendritic cell activation and skin penetration. *Journal of Investigative Dermatology* **122**, 1154–1164.
54. Rougier, N., Redziniak, G., Schmitt, D. & Vincent, C. (1998). Evaluation of the capacity of dendritic cells derived from cord blood $CD34^+$ precursors to present haptens to unsensitized autologous T cells *in vitro*. *Journal of Investigative Dermatology* **110**, 348–352.
55. Rougier, N., Redziniak, G., Mougin, D., Schmitt, D. & Vincent, C. (2000). *In vitro* evaluation of the sensitisation potential of weak contact allergens using langerhans-like dendritic cells and autologous T cells. *Toxicology* **145**, 73–82.
56. De Smedt, A.C.A., Van Den Heuvel, R.L., Bernemen, Z.N. & Schoeters, G.E.R. (2001). Modulation of phenotype, cytokine production and stimulatory function of CD34+ cell-derived DC by $NiCl_2$ and SDS. *Toxicology in Vitro* **15**, 319–325.
57. De Smedt, A.C.A., Van Den Heuvel, R.L., Van Tendeloo, V.F.I., Berneman, Z.N. & Schoeters, G.E.R. Capacity of CD34+ progenitor-derived dendritic cells to distinguish between sensitisers and irritants. *Toxicology Letters*, in press.
58. Boislève, F., Kerdine-Römer, S., Rougier-Larzat, N. & Pallardy M. (2004). Nickel and DNCB induce CCR7 expression on human dendritic cells through different signalling pathways: role of TNF-α and MAPK. *Journal of Investigative Dermatology* **123**, 494–502.
59. Boislève, F., Kerdine-Römer, S. & Pallardy, M. (2004). Implication of the MAPK pathways in the maturation of human dendritic cells induced by nickel and TNF-α. *Toxicology* **206**, 233–244..
60. Dietz, A.B., Bulur, P.A., Knutson, G.J., Matasic, R. & Vuk-Pavlovic, S. (2000). Maturation of human monocyte-derived dendritic cells studied by microarray hybridization. *Biochemical and Biophysical Research Communications* **275**, 731–738.
61. Le Naour, F., Hohenkirk, L., Grolleau, A., Misek, D.E., Lescure, P., Geiger, J.D., Hanash, S. & Beretta, L. (2001). Profiling changes in gene expression during differentiation and maturation of monocyte-derived dendritic cells using both oligonucleotide microarrays and proteomics. *Journal of Biological Chemistry* **276**, 17,920–17,931.
62. Moschella, F., Maffei, A., Catanzaro, R.P., Papadopoulos, K.P., Skerrett, D., Hesdorfer, C.S. & Harris, P.E. (2001). Transcript profiling of human dendritic cells maturation-induced under defined culture conditions: comparison of the effects of tumour necrosis factor alpha, soluble CD40 ligand trimer and interferon gamma. *British Journal of Haematology* **114**, 444–457.
63. Chen, Z., Gordon, J.R., Zhang, X. & Xiang, J. (2002). Analysis of the gene expression profiles of immature versus mature bone-marrow-derived dendritic cells using DNA arrays. *Biochemical and Biophysical Research Communications* **290**, 66–72.
64. Lindstedt, M., Johansson-Lindbom, B. & Borrebaeck, C.A.K. (2002). Global reprogramming of dendritic cells in response to a concerted action of inflammatory mediators. *International Immunology* **14**, 1203–1213.
65. Messmer, D., Messmer, B. & Chiorazzi, N. (2003). The global transcriptional maturation program and stimuli-specific gene expression profiles of human myeloid dendritic cells. *International Immunology* **15**, 491–503.
66. Türeci, O., Bian, H., Nestle, F.O., Raddrizzani, L., Rosinski, J.A., Tassis, A., Hilton, H., Walstead, M., Sahin, U. & Hammer, J. (2003). Cascades of transcriptional induction during dendritic cell maturation revealed by genome-wide expression analysis. *FASEB Journal* **17**, 836–847.

67. Ryan, C.A., Gildea, L.A., Hulette, B.C., Dearman, R.J., Kimber, I. & Gerberick, G.F. (2004). Gene expression changes in peripheral blood-derived dendritic cells following exposure to a contact allergen. *Toxicology Letters* **150**, 301–316.
68. Kühn, U., Brand, P., Willemsen, J., Jonuleit, H., Enk, A.H., van Brandwijk-Peterhans, R., Saloga, J., Knop, J. & Becker D. (1998). Induction of tyrosine phosphorylation in human MHC Class II-positive antigen-presenting cells by stimulation with contact sensitisers. *Journal of Immunology* **160**, 667–673.
69. Ardeshna, K.M., Pizzey, A.R., Devereux, S. & Khwaja, A. (2000). The PI3 kinase, p38 SAP kinase, and NF-kappaB signal transduction pathways are involved in the survival and maturation of lipopolysaccharide-stimulated human monocyte-derived dendritic cells. *Blood* **96**, 1039–1046.
70. Underhill, D.M. & Ozinsky, A. (2002). Toll-like receptors: key mediators of microbe detection. *Current Opinion in Immunology* **14**, 103–110.
71. Dunne, A. & O'Neill, L. A. (2003). The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. *Science's STKE 2003 (171)*, re3.
72. Aiba, S., Manome, H., Nakagawa, S., Mollah, Z.U., Mizuashi, M., Ohtani, T., Yoshino, Y. & Tagami, H. (2003). p38 Mitogen-activated protein kinase and extracellular signal-regulated kinases play distinct roles in the activation of dendritic cells by two representative haptens, $NiCl_2$ and 2,4-dinitrochlorobenzene. *Journal of Investigative Dermatology* **120**, 390–399.
73. Arrighi, J. F., Rebsamen, M., Rousset, F., Kindler, V. & Hauser, C. (2001). A critical role for p38 mitogen-activated protein kinase in the maturation of human blood-derived dendritic cells induced by lipopolysaccharide TNF-alpha and contact sensitisers. *Journal of Immunology* **166**, 3837–3845.
74. Bennett, B.L., Sasaki, D.T., Murray, B.W., O'Leary, E.C., Sakata, S.T., Xu, W., Leisten, J.C., Motiwala, A., Pierce, S., Satoh, Y., Bhagwat, S.S., Manning, A.M. & Anderson, D.W. (2001). SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proceedings of the National Academy of Sciences USA* **98**, 13,681–13,686.
75. Laderach, D., Compagno, D., Danos, O., Vainchenker, W. & Galy, A. (2003). RNA interference shows critical requirement for NF-kappa B p50 in the production of IL-12 by human dendritic cells. *Journal of Immunology* **15**, 1750–1757.
76. Luft, T., Jefford, M., Luetjens, P., Toy, T., Hochrein, H., Masterman, K.A., Maliszewski, C., Shortman, K., Cebon, J. & Maraskovsky, E. (2002). Functionally distinct dendritic cell (DC) populations induced by physiologic stimuli: prostaglandin E(2) regulates the migratory capacity of specific DC subsets. *Blood* **100**, 1362–1372.
77. Scandella, E., Men, Y., Gillessen, S., Forster, R., & Groettrup, M. (2002). Prostaglandin E2 is a key factor for CCR7 surface expression and migration of monocyte-derived dendritic cells. *Blood* **100**, 1354–1361.
78. Bour, H., Peyron, E., Gaucherand, M., Garrigue, J.L., Desvignes, C., Kaiserlian, D., Revillard, J.P. & Nicolas, J.F. (1995). Major histocompatibility complex class I-restricted CD8+ T cells and class II-restricted CD4+ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene. *European Journal of Immunology* **25**, 3005–3010.
79. Anderson, C., Hehr, A., Robbins, R., Hasan, R., Athar, M., Mukhtar, H. & Craig, E. (1995). Metabolic requirements for induction of contact hypersensitivity to immunotoxic polyaromatic hydrocarbons. *Journal of Immunology* **155**, 3530–3535.
80. Xu, H., Dilulio, N.A. & Fairchild, R.L. (1996). T cell populations primed by hapten sensitisation in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon gamma-producing (Tc1) effector CD8+ T cells and interleukin (IL) 4/IL-10-producing (Th2) negative regulatory CD4+ T cells. *Journal of Experimental Medicine* **183**, 1001–1012.
81. Moulon, C., Wild, D., Dormoy, A. & Weltzien, H.U. (1998). MHC-dependant and independent activation of human nickel-specific CD8+ cytotoxic T cells from allergic donors. *Journal of Investigative Dermatology* **111**, 360–366.
82 Hauser, C. & Katz, S.I. (1990). Generation and characterization of T-helper cells by primary *in vitro* sensitisation using Langerhans cells. *Immunological Reviews* **117**, 67–84.
83. Moulon, C., Peguet-Navarro, J., Courtellemont, P., Redziniak, G. & Schmitt, D. (1993). *In vitro* primary sensitisation and restimulation of hapten-specific T cells by fresh and cultured epidermal Langerhans' cells. *Immunology* **80**, 373–379.
84. Krasteva, M., Peguet-Navarro, J., Moulon, C., Courtellemont, P., Redziniak, G. & Schmitt, D. (1996). *In vitro* primary sensitisation of hapten-specific T cells by cultured human epidermal Langerhans cells: a screening predictive assay for contact sensitisers. *Clinical and Experimental Allergy* **26**, 563–570.
85. Streilein, J.W. & Grammer, S.F. (1989). *In vitro* evidence that Langerhans cells can adopt two functionally distinct forms capable of antigen presentation to T lymphocytes. *Journal of Immunology* **143**, 3925–3933.
86. Puré, E., Inaba, K. & Crowley, M.T. (1990). Antigen processing by epidermal Langerhans cells correlates with the level of biosynthesis of major histocompatibility complex class II molecules and expression of invariant chain. *Journal of Experimental Medicine* **172**, 1459–1469.
87. Romani, N., Gruner, S., Brang, D., Kampgen, E., Lenz, A., Trockenbacher, B., Konwalika, G., Fritsch, P.O., Steinman, R.M. & Schuler, G. (1994). Proliferating dendritic cell progenitors in human blood. *Journal of Experimental Medicine* **180**, 83–93.
88. Caux, C., Massacrier, C., Dezutter-Dambuyant, C., Vanbervliet, B., Jacquet, C., Schmitt, D. & Banchereau, J. (1995). Human dendritic cells Langerhans cells generated *in vitro* from CD34+ progenitors can prime naïve CD4+ T cells and process soluble antigen. *Journal of Immunology* **155**, 5427–5435.
89. Rustemeyer, T., De Ligter, S., Von Blomberg, B.M., Frosch, P.J. & Scheper, R.J. (1999). Human T lymphocyte priming *in vitro* by haptenated autologous dendritic cells. *Clinical and Experimental Immunology* **117**, 209–216.
90. Dai, R. & Streinlein, J.W. (1998). Naïve, hapten-specific human T lymphocytes are primed *in vitro* with derivatized blood mononuclear cells. *Journal of Investigative Dermatology* **110**, 29–33.
91. Guironnet, G., Dalbiez-Gauthier, C., Rousset, F., Schmitt, D. & Peguet-Navarro, J. (2000). *In vitro* human T cell sensitisation to haptens by monocyte-derived dendritic cells. *Toxicology in Vitro* **14**, 517–522.
92. Opdyke, D.L.J. (1975). Monograph on fragrance raw

material. *Food and Cosmetics Toxicology* **13**, 681–923.
93. De Groot, A.C., Liem, D.H., Nater, J.P. & van Ketel, W.G. (1985). Patch tests with fragrance materials and preservatives. *Contact Dermatitis* **12**, 87–92.
94. Larsen, W., Nakayama, H., Lindberg, M., Fisher, T., Elsner,P., Burrows, D., Jordan, W., Shaw, S., Wilkinson, J., Marks, J.Jr, Sugawara, M. & Nethercott J. (1996). Fragrance contact dermatitis: a worldwide multicenter investigation (Part I). *American Journal of Contact Dermatitis* **7**, 77–83.
95. Larsen, W.G. (2002). Fragrance testing in the 21st century. *Contact Dermatitis* **46**, 60–61.
96. Johansen, J.D., Andersen, T.F., Kjoller, M., Veien, N., Avnstorp, C., Andersen, K.E. & Menne, T. (1998). Identification of risk products for fragrance contact allergy: a case-referent study based on patient's histories. *American Journal of Contact Dermatitis* **9**, 80–86.
97. Goodwin, B.J.F., Crevel, R.W.R. & Johnson, A.W. (1981). A comparison of three guinea pig sensitisation procedures for the detection of 19 reported human contact sensitisers. *Contact Dermatitis* **7**, 248–258.
98. Marzulli, F.N. & Maibach, H.I. (1974). The use of graded concentrations in studying skin sensitisers: experimental contact sensitisation in man. *Food and Cosmetics Toxicology* **12**, 219–227.
99. Basketter, D.A. & Scholes, E.W. (1992). Comparison of the local lymph node assay with the guinea pig maximization test for the detection of a range of contact allergens. *Food and Cosmetics Toxicology* **30**, 65–69.
100. Basketter, D.A. & Liden, C. (1992). Further investigation on the prohapten concept: reactions to benzene derivative in man. *Contact Dermatitis* **27**, 90–97.
101. Régnier, M., Staquet, M.J., Schmitt, D. & Schmidt, R. (1997). Integration of Langerhans cells into a pigmented reconstructed human epidermis. *Journal of Investigative Dermatology* **109**, 510–512.
102. Sivard, P., Dezutter-Dambuyant, C., Kanitakis, J., Mosnier, J.F., Hamzeh, H., Bechetoille, N., Berthier, O., Sabido, O., Schmitt, D., Genin, C. & Misery, L. (2003). *In vitro* reconstructed mucosa-integrating Langerhans cells. *Experimental Dermatology* **2**, 346–355.
103. Ayehunie, S., Cannon, C.L., Lamore, S., Kubilus, J., LaBonte, J., Pudney, J., Anderson, D., Sodroski, J. & Klausner, M. (2002). Organotypic ectocervico-vaginal tissue model containing dendritic cells. *Microbicide 2002 Meeting*, Antwerp, Belgium, 12–15 May 2002, 9pp. Website http://www.cdnaids.ca/web/mailouts.nsf/0/85f80b6904867adb85256c62006522e0/$FILE/ReportMicrobiocides2002.pdf (Accessed 24.2.05).
104. Facy, S., Flouret, V., Regnier, M. & Schmidt, R. (2004). Langerhans cells integrated into human recontructed epidermis respond to known sensitisers and ultraviolet exposure. *Journal of Investigative Dermatology* **122**, 552–553.
105. Schempp, C.M., Dittmar, H.C., Hummier, D., Simon-Haarhaus, B., Schulte-Monting, J., Schopf, E. & Simon, J.C. (2000). Magnesium ions inhibit the antigen-presenting function of human epidermal Langerhans cells *in vivo* and *in vitro*. Involvement of ATPase, HLA-DR, B7 molecules, and cytokines. *Journal of Investigative Dermatology* **115**, 680–686.
106. Larsen, C.P., Steinman, R.M., Witmer-Pack, M., Hankins, D.F., Morris, P.J. & Austyn, J.M. (1990). Migration and maturation of Langerhans cells in skin transplants and explants. *Journal of Experimental Medicine* **172**, 1483–1493.
107. Herouet, C., Cottin, M., Leclaire, J., Enk, A. & Rousset, F. (2000). Contact sensitisers specifically increase MHC class II expression on immature dendritic cells. *In Vitro and Molecular Toxicology* **13**, 113–123.
108. St Louis, D.C., Woodcock, J.B., Fransozo, G., Blair, P.J., Carlson, L.M., Murillo, M., Wells, M.R., Williams, A.J., Smoot, D.S., Kaushal, S., Grimes, J.L., Harlan, D.M., Chute, J.P., June, C.H., Siebenlist, U. & Lee, K.P. (1999). Evidence for distinct intracellular signaling pathways in CD34+ progenitor to dendritic cell differentiation from a human cell line model. *Journal of Immunology* **162**, 3237–3248.
109. Ashikaga, T., Hoya, M., Itagaki, H., Katsumura, Y. & Aiba, S. (2002). Evaluation of CD86 expression and MHC class II molecule internalization in THP-1 human monocyte cells as predictive endpoints for contact sensitisers. *Toxicology in Vitro* **16**, 711–716.
110. Yoshida, Y., Sakaguchi, H., Ito, Y., Okuda, M. & Suzuki, H. (2003). Evaluation of the skin sensitisation potential of chemicals using expression of costimulatory molecules, CD54 and CD86, on the naive THP-1 cell line. *Toxicology in Vitro* **17**, 221–228.
111. Koss, A., Lucero, G. & Koziner, B. (1996). Granulocyte-colony stimulating factor, granulocyte-macrophage colony stimulating factor and interleukin 4 induce differentiation in the U-937 human monocytic leukemia cell line. *Leukemia and Lymphoma* **22**,163–171.
112. Tenno, T., Oberg, F., Nilsson, K. & Siegbahn, A. (1997). Induction of differentiation in U-937 and NB4 cells is associated with inhibition of tissue factor production. *European Journal of Haematology* **63**, 112–119.
113. OECD (2002). *OECD Guidelines for the Testing of Chemicals, No. 429, The Local Lymph Node Assay*, 7pp. Paris, France: Organization for Economic Cooperation and Development.
114. Ryan, C.A., Cruse, L.W., Skinner, R.A., Dearman, R.J., Kimber, I. & Gerberick, G.F. (2002). Examination of a vehicle for use with water soluble materials in the murine local lymph node assay. *Food and Chemical Toxicology* **40**, 1719–1725.
115. Gerberick, G.F., Ryan, C.A., Kimber, I., Dearman, R.J., Lea, L.J. & Basketter, D.A. (2000). Local lymph node assay validation assessment for regulatory purposes. *American Journal of Contact Dermatitis* **11**, 3–18.
116. NIH (1999). *The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds*. NIH No. 99-4494. 211pp. Bethesda, MD, USA: National Institutes of Health.
117. Gerberick, G.F., Robinson, M.K., Ryan, C.A., Dearman, R.J., Kimber, I., Basketter, D.A., Wright, Z.M. & Marks, J.G. (2001). Contact allergenic potency: correlation of human and local lymph node assay data. *American Journal of Contact Dermatitis* **12**, 156–161
118. Smith, C.K. & Hotchkiss, S.A. (2001). *Allergic Contact Dermatitis. Chemical and Metabolic Mechanisms*, 310pp. London, UK: Taylor & Francis.
119. Smith-Pease, C.K., Basketter, D.A. & Patlewicz, G.Y. (2003) Contact allergy: the role of skin chem-

istry and metabolism. *Clinical and Experimental Dermatology* **2**, 1777–1783.
120. Basketter, D.A., Scholes, E.W., Evans, C.D. & Kimber, I. (1992). Divergent responses to sulphanilic acid in the guinea pig maximization test and the local lymph node assay. *Contact Dermatitis* **27**, 209–213.
121. Wahlberg, J.E. (1989). Nickel: animal sensitisation assays. Nickel and skin. In *Immunobiology and Toxicology* (ed. H.I. Maibach & T. Menné), pp. 65–74. Boca Raton, FL, USA: CRC Press.
122. Basketter, D.A., Lea, L., Cooper, K., Ryan, C.J., Gerberick, G.F., Dearman, R.J. & Kimber, I. (1999). The identification of metal allergens in the local lymph node assay. *American Journal of Contact Dermatitis* **10**, 207–212.
123. Lepoittevin, J-P., Basketter, D.A., Dooms-Goossens, A. & Karlberg, A-T. (1997). *Allergic Contact Dermatitis: The Molecular Basis*, 186pp. Heidelberg, Germany: Springer-Verlag.
124. Thierse, H.J., Moulon, C., Allespach, Y., Zimmermann, B., Doetze, A., Kuppig, S., Wild, D., Herberg, F. & Weltzien, H.U. (2004). Metal-protein complex-mediated transport and delivery of Ni2+ to TCR/MHC contact sites in nickel-specific human T cell activation. *Journal of Immunology* **172**, 1926–1934.
125. Basketter, D.A., Pease Smith, C.K. & Patlewicz, G.Y. (2003). Contact allergy: the local lymph node assay for the prediction of hazard and risk. *Clinical and Experimental Dermatology* **28**, 218–221.
126. Felter, S.P., Ryan, C.A., Basketter, D.A. & Gerberick, G.F. (2003). Application of the risk assessment paradigm to the induction of allergic contact dermatitis. *Regulatory Toxicology and Pharmacology* **37**, 1–10.
127. Dearman, R.J., Smith, S., Basketter, D.A. & Kimber, I. (1997). Classification of chemical allergens according to cytokine secretion profiles of murine lymph node cells. *Journal of Applied Toxicology* **17**, 53–62.