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Restraint-Induced Modification of Langerhans Cell Morphology and Migration in BALB/c Mice is Dependent Upon Timing of Restraint

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The timing of a stressful event with respect to antigen exposure affects the development of the immune response. We have reported that restraint applied to a naive mouse prior to sensitization (day 1) decreased chemical-induced changes in ear swelling and lymphocytosis and restraint applied during sensitization (days 1 and 2) or prior to challenge (day 6), increased ear swelling. We hypothesized that these dichotomous effects of restraint would be reflected in changes in Langerhans cell (LC) migration and morphology. To compare the effects of restraint on LC in naive and DNFB-treated mice, we sensitized mice onto the back with 0.5% DNFB on days 1 and 2 and challenged onto the ears with 0.25% DNFB on day 6. Mice were restrained for 2 h immediately prior to chemical application on day 1 or on day 6 prior to challenge. LC morphology and migration were examined in epidermal sheets by counting the number of FITC-conjugated Ia stained cells per mm^2 at 24 h after DNFB-challenge. To assess the effect of restraint on T cell proliferation *in vivo*, 24 h following challenge, T lymphocyte proliferation was examined using the local lymph node assay. We determined that DNFB induced a significant reduction in the number of epidermal LC at 24 h. Restraint on day 1 resulted in the retention of LC in the epidermis and the LC had longer dendritic processes than nonrestrained mice. However, LC from mice restrained on day 6 demonstrated a dendritic cell like-morphology similar to nonrestrained mice but showed a significant reduction in epidermal LC numbers 24 h after DNFB challenge. Furthermore, we demonstrated a significant two-fold decrease in T cell proliferation in mice restrained on day 1 and an elevation of T cell proliferation in mice restrained on day 6. These data suggest that acute restraint stress applied prior to sensitization modulates epidermal LC morphology and migration and T cell proliferation differently than restraint applied prior to challenge.

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Intact Lymphoid Structure and Germinal Center Formation are Not Crucial for Mounting an Antigen Specific Humoral Response in CD18 Null Mice

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β-2 integrins play a central role in host defence and if specifically mutated lead to a disorder known as leukocyte adhesion deficiency 1 (LAD1) in humans. Mice with a CD18 null mutation suffer from recurrent bacterial infections, impaired wound healing and skin ulcers closely resembling human LAD1. We here report on the impact of CD18 deficiency on the organization of lymphoid tissue, B cell differentiation, germinal center formation and T-dependent B cell response. CD18 deficient and wildtype (wt) control mice were immunized with the T-dependent antigen nitrophenylacetate (NP) chicken gammaglobulin to analyze NP hapten-specific antibody production. All animals were re-injected with the soluble antigen at day 34 to further study the secondary immune response. The detection of serum NP-specific antibodies showed only a slightly retarded and decreased primary response in in CD18 null mice, whereas class switch and affinity maturation occurred as in wt mice. In the secondary immune response virtually no difference to wt controls existed indicating a normal memory B cell generation and function. These results were completely unexpected, as conventional histology and immunohistochemistry with stainings for proliferation (Ki-67), germinal center B cells (peanut agglutinin), T-cell and dendritic cell markers of all major lymphoid tissues which were performed at three time points after primary immunization had revealed a completely resolved architecture of the lymphoid tissues without any initiation of classical germinal center reactions. In ultrastructural analysis the tight membrane adhesions (< 5 nm) between lymphocytes and dendritic cells observed in wt mice (n=6) were missing in CD18 null mutants (n=6) in all lymphoid tissue sections analyzed (lymph nodes, spleen, Peyer's patches, tonsils). However, rudimentary bridges of membrane contacts existed. We here provide evidence that formerly described lymphoid structures and germinal center formation are not necessarily required to mount an antigen specific immune response in CD18 null mice, but that residual structures can compensate. (BMBF 01 KS 9502)

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Rho GTPases are Key Regulators of Dendritic Cell Morphology and Function

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Dendritic cells (DCs) are characterized by their irregular, dendrite (i.e. lamellipodium and filopodium)-bearing plasma membrane and by their unique ability to activate naïve T lymphocytes. The latter function is linked to their high migratory capacity and their abundant display of MHC and costimulatory molecules. Interestingly, cell shape, migration, and export of MHC products to the cell surface of DCs require actin assembly. Molecules that mediate actin polymerization, and, thus, may control DC phenotype and function are small Rho GTPases. To study the importance of individual GTPases in DCs, stem cell-derived Langerhans cell-type DCs were transfected with constructs encoding myc-tagged dominant negative mutants of Cdc42 (N17-Cdc42), Rac1 (N17-Rac1) or RhoA (N14-RhoA). CD40 ligand-stimulated DC transfectants showed stable expression of the myc-tagged transgenes over several days and survived at similar rates as empty vector transfected control cells. By confocal microscopy, we observed dramatic alterations of the cellular cytoskeleton imposed by the various constructs. N17-Cdc42+ DCs were essentially round without filopodia or lamellipodia and displayed an only poorly developed actin cytoskeleton. N17-Rac1+ and N14-RhoA+ DCs displayed collapsed lamellipodia and thinned surface-associated actin, respectively. In contrast, constitutively active GTPase mutants induced lamellipodia formation (L61-Rac1), short membrane protrusions (L63-RhoA) and, most strikingly, long, actin-bearing dendrites/filopodia (L61-Cdc42) in monocytes. Thus, by phenotype, these monocytes were hardly discernable from DCs. Furthermore, DCs transfected with dominant negative mutants of Rho-GTPases showed reduced migration to chemokine stimuli and were also impaired in their ability to stimulate T cells. Surprisingly, DCs require functional Cdc42 for the stimulation of CD8+, but less so, for CD4+ T cells. Apparently, this effect is mediated by reduced MHC class I surface export of this molecule from (post)Golgi compartments but is not due to destabilization of surface MHC class I. In contrast, MHC class II surface expression and IL-12 secretion were hardly affected. In summary, DC morphology and DC function are co-ordinately controlled by similar, if not identical, signaling modules, i.e. Rho GTPases. Moreover, Cdc42 seems to be of particular importance for DC-T cell communication and, thus, for T-cell-dependent immunity.

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IL-10-Treated Human Dendritic Cells Induce Anergic CD4+ and CD8+ T Cells with Antigen-Specific Suppressor Activity

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Recently, we demonstrated that IL-10-treated dendritic cells (DC) induce an alloantigen- or peptide-specific anergy in various CD4+ and CD8+ T cell populations. In this study we wanted to analyse whether these anergic T cells have immunoregulatory functions. Coculture experiments revealed that alloantigen-specific anergic CD4/8+ T cells suppress the proliferation of naive syngeneic T cells in a dose dependent fashion. The same effect was observed if tyrosinase-specific cytotoxic CD8+ T cells or the haemagglutinin-specific CD4+ T cell clone HA1.7 were cocultured with anergic T cells of the same specificity. In contrast, anergic T cells did not mediate an antigen-independent bystander inhibition. Functional analysis revealed that previously activated tyrosinase-specific CD8+ T cells cocultured with anergic tyrosinase-specific CD8+ T cells failed to lyse a tyrosinase-expressing melanoma cell line. Suppression was dependent on cell to cell contact between anergic and responder T cells and required activation by antigen-loaded DC. Supernatants of anergic T cells did not mediate T cell suppression. Inhibition could be overcome by addition of IL-2 or anti-CD3-/CD28-mAb and was blocked by CTLA-4-Ig or anti-CD86-mAb. Furthermore, anergic T cells displaying increased expression of CTLA-4 surface molecules were involved in the suppressor activity. Taken together, our experiments demonstrate that anergic T cells induced by coculture with IL-10-treated DC mediate antigen-specific suppression of T cells. Induction of anergic T cells might be exploited therapeutically for suppression of cellular immune responses in allergic or autoimmune diseases with identified (auto-) antigen.

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Adhesion of $CD34^+$-Derived Dendritic Cells to Human Dermal Microvascular Endothelial Cells is Down-Regulated upon Maturation and Depends on CD11a, CD11b, CD36

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Dendritic cells (DC) function as sentinels of the immune system. When bone marrow-derived DC precursors reach their target tissue, they need to bind and migrate out of *microvascular* endothelial cells, in particular. To determine the adhesive properties of DC at various differentiation stages to human dermal microvascular endothelial cells (HDMEC), DC were generated from human cord blood-derived $CD34^+$ hematopoietic progenitors by culture in the presence of GM-CSF, TNF-α and hSCF. To enhance DC maturation, cells were additionally exposed to a defined cocktail of IL-1β (2 ng per ml)/IL-6 (1000 U per ml)/TNF-α (10 ng per ml)/PGE2 (1 μg per ml) ("cocktail DC"). Adhesion was assessed and quantified by phase contrast microscopy and by means of a fluorimetric adhesion assay. Significantly more immature (37.8 ± 9.7%) than mature DC (13.8 ± 6.3% for conventionally matured DC and 2.5 ± 0.87% for "cocktail DC") bound to unstimulated HDMEC. HDMEC pretreatment with TNF-α and IFN-γ resulted in an enhanced attachment of both immature and mature DC by 50–160%. Flow cytometric analysis revealed that mature DC are characterized by the lack of CD31, CD36, CD45RA and CLA expression and by the low expression of CD11a, CD11b and CD49d. Blocking mAb's against CD11a, CD11b, and CD36 markedly inhibited DC binding (mean inhibition 65–70%), whereas anti-CD49d Ab's did not. Simultaneous application of these mAb's did not potentiate inhibition. Our data support the hypothesis of immunosurveillance with selective recruitment of immature blood DC at sites of cutaneous inflammation. They might have potential relevance in the development of immunotherapy strategies for cancer indicating the inefficacy of intravenous DC application.

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Differential Effects of LPS and TGF-β on the Production of IL-12 p40 and IL-6 of Mouse Langerhans Cells, Spleen-Derived Dendritic Cells and Macrophages

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The response of Langerhans cells (LC)/Dendritic cells (DC) to bacterial lipopolysaccaride (LPS) is the best example of innate recognition. However, contradictory results have been reported on the ability of LC/DC to respond to LPS. Here, we examined modulatory effects of LPS on IL-12 p40 and IL-6 production of highly purified LC (95%), spleen-derived $CD11c^+$ DC and peritoneal exudate macrophages (Mφ) by ELISA. Low dose LPS (1 ng per ml) up-regulated IL-12 p40 production of anti-CD40/IFN-γ-stimulated Mφ, and it also up-regulated IL-6 production of anti-CD40/IFN-γ-stimulated DC and Mφ. In contrast, low dose LPS up-regulated neither IL-12 p40 nor IL-6 production of stimulated LC. Since LC did not express CD14 and spleen-derived $CD11c^+$ DC expressed low density of CD14, it is likely that LC, DC and Mφ respond differently to low dose LPS depending on the density of cell surface CD14 expression, which is at least known to be involved in LPS-induced Mφ activation. However, high dose LPS (1 μg per ml) up-regulated IL-6 production of LC, suggesting the ability of LC to respond to LPS. We also explored the immunomodulatory effects of transforming growth factor (TGF)-β on stimulated LC and Mφ, since TGF-β is known to inhibit signalling of LPS-induced inflammatory responses, especially in Mφ. As has been expected, TGF-β_1 down-regulated IL-12 p40 and IL-6 production of anti-CD40/IFN-γ/LPS-stimulated Mφ. TGF-β_1 up-regulated IL-12 p40 production of anti-CD40/IFN-γ-stimulated Mφ, but did not modify IL-6 production of anti-CD40/IFN-γ-stimulated Mφ. In contrast to Mφ, TGF-β_1 up-regulated IL-6 production of anti-CD40/IFN-γ/LPS (1 μg per ml)-stimulated LC, and also up-regulated IL-6 and IL-12 p40 production of anti-CD40/IFN-γ-stimulated LC. These results suggest that TGF-β is the key cytokine in the skin innate immunity by up-regulating LC secretion of IL-12 and IL-6, which are essential for activating cell-mediated and humoral immunity.