

Cellular dynamics in the draining lymph nodes during sensitization and elicitation phases of contact hypersensitivity

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Background: The different role of various immunological effector cells in contact hypersensitivity (CHS) is receiving increased attention. During the past decade, the involvement of different cell types in CHS has been investigated by the use of antibody-induced depletion of specific subtypes of immunological cells and by studying knockout mice lacking one or more of these immunological cell populations.

Objectives: To develop a method for studying the collective cellular dynamics of immune cells in the draining lymph nodes during CHS in intact animals.

Patients/Methods: Mice were sensitized and/or challenged with 2,4-dinitrofluorobenzene or oxazolone. Using multi-parameter flow cytometry we determined the proliferation, activation state, and absolute number of helper T cells, cytotoxic T cells, B cells, and natural killer cells in the draining lymph nodes.

Results: The presented method can be applied to evaluate the effect of different contact allergens on various cell populations of the immune system.

Conclusions: Our study support recent findings that several cell types seem to be involved in CHS.

Key words: activation; contact hypersensitivity; flow cytometry; local lymph node assay; lymphocytes. © Blackwell Munksgaard, 2007.

Accepted for publication 7 June 2007

The immune response responsible for contact hypersensitivity (CHS) is generally considered as a delayed-type hypersensitivity reaction primarily mediated by T cells. During the past decade, the role of various cell types involved in the reaction has been investigated by the use of antibody-induced cell depletion and different types of knockout mice (1–7). Early studies have shown a key role of cytotoxic CD8⁺ T cells in both the sensitization and elicitation phases of CHS (1–3). In contrast, CD4⁺ T cells have been suggested to have a regulatory or inhibitory role in the response, as lack of CD4⁺ T cells results in an increased response to contact allergens (2, 8). Studies using B cell knockout mice show that B cells are not required for developing CHS

(4, 6). However, a lack of B cells resulted in reduced CHS responses (4–6). Recently, O’Leary et al. (7) have investigated the role of natural killer (NK) cells in CHS. Using mice devoid of T and B cells, they showed that NK cells can mediate long-lived, antigen-specific adaptive recall responses to 2,4-dinitrofluorobenzene (DNFB) and oxazolone (OXA) independent of T and B cells. Thus, the underlying immunological reaction behind CHS involves various cell types, which altogether may contribute to the pathogenesis of CHS.

The most commonly used method for testing the relative potency of skin-sensitizing chemicals is the mouse local lymph node assay (LLNA) (9). In LLNA, the potency of a chemical to act as an allergen is determined by unspecific cell proliferation

in the draining lymph node cells as measured by the incorporation of radiolabelled thymidine (9, 10). Thus, this assay does not allow for determination of accumulation, activation, and proliferation of specific immune cellular subpopulations. Knowledge of the cellular dynamics in the draining lymph nodes during the sensitization and elicitation phases of CHS will broaden our understanding of the pathogenesis of CHS and may show important pathophysiological aspects of CHS that will be important for the development of new treatments.

In this study, we present a method to study the cellular dynamics in the draining lymph nodes during CHS. Using the sensitization protocol from the LLNA together with multi-parameter flow cytometry and 5-bromo-2-deoxyuridine (BrdU) incorporation, we determined the kinetics of accumulation and proliferation of CD4⁺ and CD8⁺ T cells, B cells, and NK cells, as well as cell surface activation markers on T cells. These parameters could be determined during both the sensitization and elicitation phases of CHS. Our data show that this method can be applied to evaluate the effect of different contact allergens on various cellular subpopulations of the immune system and support recent findings that not only T cells, but also B cells, and NK cells seem to be involved in CHS.

Materials and Methods

Mice

Female C57Bl/6 mice were purchased from Taconic (Ry, Denmark) and housed in specific pathogen-free facilities at the Department of Experimental Medicine, Panum Institute in accordance with national animal-protection guidelines. We chose to study C57Bl/6 mice as this mouse strain is most commonly used for making gene-modified mice. Thus, in later studies modified C57Bl/6 mice would be an obvious choice to analyse the role of various components of the immune system in CHS. Animal welfare was inspected regularly by facility veterinarians, and experimental procedures were approved by the national animal ethics committee (permission number 2002/561-540). Mice used for experiments were 8–12 weeks old.

Chemicals

DNFB (Sigma, Broendby, Denmark) and OXA (Sigma) were prepared in acetone at a concentration of 0.15% and 0.3%, respectively, and acetone was used as vehicle control. When stated BrdU (Sigma) were diluted to a final concentration of 0.8 mg/ml in the drinking water.

Hapten sensitization and challenge to elicit CHS

For sensitization, mice were painted with DNFB or OXA on the dorsal side of both ears for 3 consecutive days (D0–2). Mice were euthanized and the draining auricular lymph nodes were removed on D3, D5, D7, and D10. When the ear thickness had returned to the level of control mice and skin showed no clinical signs of dermatitis mice were challenged on both ears with 1 dose of DNFB or OXA. Mice were euthanized and the draining auricular lymph nodes were removed on D0, D1, D2, and D4 after challenge. 2 days before euthanization, BrdU was added to the drinking water. Single-cell suspensions were prepared from the draining auricular lymph nodes. The cells were counted and subsequently examined for expression of various markers by the use of flow cytometry. The increment in ear thickness was measured in a blinded manner at 1–2 day intervals during both sensitization and challenge using an engineer's micrometer (Mitutoyo, Tokyo, Japan) and shown as percentages. The magnitude of ear swelling is given as the mean ear thickness in each group of 3 individual animals (i.e. 6 ears) relative to the ear thickness in unsensitized mice $\pm$ SEM.

Cell preparation and flow cytometry

Single-cell suspensions were prepared from the draining auricular lymph nodes using manual tissue dissociation on 70 μ m cell strainers (BD Biosciences, Broendby, Denmark). To determine the expression of various cell surface markers cells were incubated with fluorochrome-conjugated anti-CD4 (RM4-5), anti-CD8a (53-6.7), anti-CD19 (ID3), anti-NK1.1 (PK136), anti-CD25 (PC61), anti-CD44 (IM7), anti-CD62L (MEL-14), or anti-CD11a (2D7) monoclonal antibodies (mAb), all from BD Pharmingen. The cells were adjusted to 5×10^6 cells/ml and plated in 96-well plates (200 μ l/well). Next, the cells were incubated with the indicated mAb diluted in ice-cold phosphate-buffered saline containing 2% fetal calf serum and 0.1% NaN₃ for 30 min on ice. Finally, the cells were analyzed on a FACSCalibur (Becton Dickinson) with CELLQUEST software. Proliferating cells were identified by intracellular BrdU staining using the BrdU flow kit from BD Pharmingen according to the manufactures instructions. In brief, the cells were adjusted to 5×10^6 cells/ml and plated in 96-well plates (200 μ l/well). The cells were stained for surface expression as described above. Subsequently, the cells were fixed and permeabilized according to manufactures instructions. The cells were then treated with 300 μ g/ml DNase for 30 min at 37°C. Finally,

the cells were stained intracellular with anti-BrdU and analyzed by flow cytometry. As controls, we used the same staining protocols in cells from naïve, untreated mice.

Results

Accumulation of specific cell populations in the draining lymph nodes during the sensitization and elicitation phase of CHS

In order to determine the accumulation of specific lymphocyte populations in the draining lymph nodes during the sensitization and elicitation phases of CHS, we treated mice with 0.15% DNFB diluted in acetone or with acetone alone at the dorsum of both ears daily for 3 consecutive days. On various days, groups of mice were euthanized and single-cell suspensions were made from the draining lymph nodes. The absolute cell number was determined and the percentages of CD4⁺ and CD8⁺ T cells, B cells, and NK cells were analysed by flow cytometry. As shown in Fig. 1a–c and e the number of CD4⁺ and CD8⁺ T cells, B cells, and the absolute cell number peaked 5 days

after primary allergen application, where after it rapidly declined. In contrast, already 3 days after primary allergen application the number of NK cells reached maximum and was maintained at this level until D8 (Fig. 1d). As seen in Fig. 1c, CD19⁺ B cells showed the greatest increase in absolute numbers. Increment in ear thickness followed the same kinetics as accumulation of T and B cells in the draining lymph nodes (Fig. 1f). On D13, the cell number and occurrence of cell subpopulations in the draining lymph nodes and the ear thickness had returned to pretreatment levels (data not shown).

To investigate cell accumulation during the elicitation phase of CHS previously sensitized mice were challenged with 1 dose of DNFB on the dorsum of both ears. Groups of mice were subsequently euthanized on D0, D1, D2 and D4 after challenge. A massive accumulation of all 4 lymphocyte subpopulations was observed already at D1 (Fig. 1e). However, whereas the number of CD4⁺ and CD8⁺ T cells remained high for the rest of the observation period, the number of B and NK cells started to decline already 2 days after the challenge.

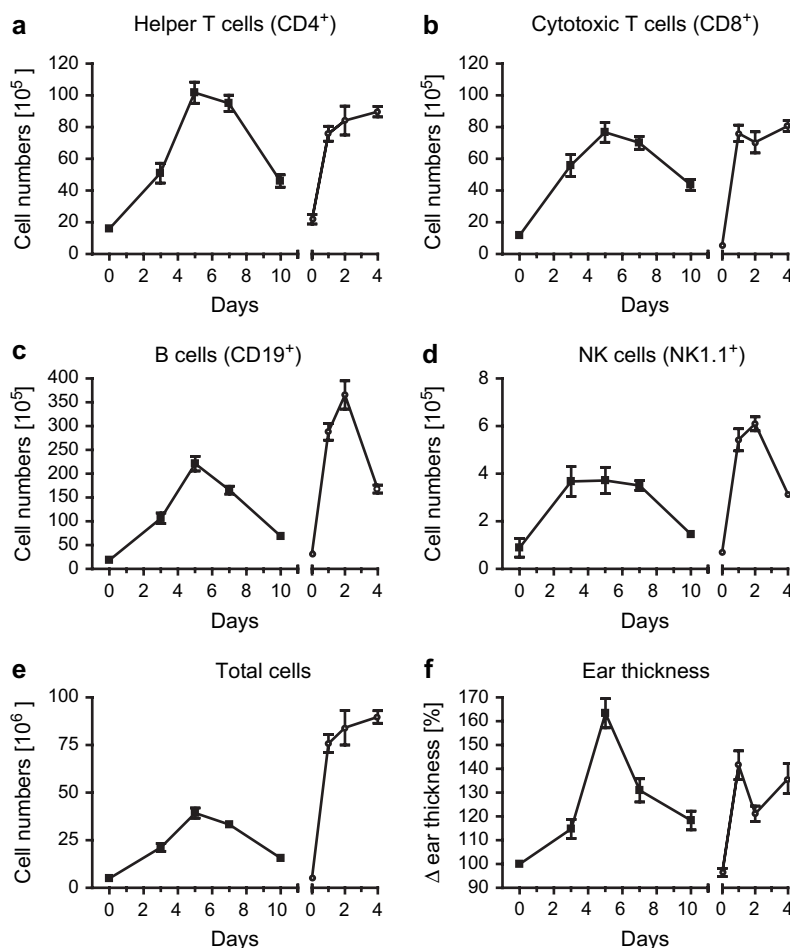


Fig. 1. Cell infiltration in the local lymph node during DNFB-induced CHS. C57BL/6 mice were painted for 3 consecutive days with 0.15% DNFB, and 1, 3, 5, and 8 days later suspensions from draining auricular lymph nodes were made and cells were counted (e) and stained for CD4 (a), CD8 (b), CD19 (c) and NK1.1 (d) expression and analysed by flow cytometry. Furthermore, groups of sensitized mice were challenged with 0.15% DNFB, and 1, 2, and 4 days post-challenge draining auricular lymph node suspensions were stained and analysed as above. During both sensitization and elicitation the ear thickness was followed (f). $n = 3-4$ mice per group from 2 independent experiments. ■ and ○ shows the kinetics of the sensitization phase and of the elicitation phase, respectively. CHS, contact hypersensitivity; DNFB, 2,4-dinitrofluorobenzene.

Proliferation of specific cell populations in the draining lymph nodes during the sensitization and elicitation phase of CHS

The absolute number of lymphocytes in the draining lymph nodes increased approximately 5 times during the sensitization and elicitation phases. This increase could be caused by an increased influx, proliferation, or reduced output of various lymphocytes subpopulations or a combination of the three. We next asked whether it would be possible to determine the fraction of cells for each specific cell population that proliferated in the draining lymph node at various time-points during the sensitization and elicitation phases of CHS. To this end, we added BrdU to the drinking water 2 days before euthanization of the mice. BrdU is a synthetic analogue of thymidine containing bromide, which can be used in the detection of proliferating cells in living tissues. BrdU is incorporated into the newly synthesized DNA of replicating cells during the S phase of the cell cycle substituting for thymidine. Thus, BrdU incorporation is analogue to H^3 -thymidine incorporation as used in the traditional LLNA. However, antibodies specific for BrdU can be used to detect cells that have incorporated BrdU and thereby it is possible to specifically identify cells that have started cell cycling. During the sensitization phase, we noticed a clear peak in BrdU⁺ cells in both the CD4⁺ and CD8⁺ T cell population at D5 (Fig. 2a and c). Approximately 21% of the CD4⁺ T cell population and 16% of the CD8⁺ T cell population in the draining lymph nodes had started proliferating at D5 (Fig. 2b and d). B cells entered cell cycling with a slower kinetics and peaked later than the T cells, which is in agreement with the need of T cell help to induce B cell activation (Fig. 2e). Approximately 6% of the B cells in the draining lymph nodes had started cell cycling at D7 (Fig. 2f). In contrast, NK cells reached maximum cell cycling already at D3 and maintained this level until D7 (Fig. 2g). Approximately 14% of the NK cells were cycling from D3 to D7 (Fig. 2h). Thus, compared with the LLNA, which gives an overall unspecific picture of cell cycling, our method further allows for determination of the specific contribution of each cell population to the cycling pool of cells. We found that during the sensitization phase of CHS CD8⁺ and CD4⁺ cells makes the highest contribution to the cycling cell pool at D3. CD4⁺ T cells dominate at D5 and B cells at D7 (Fig. 2i).

During the elicitation phase, CD4⁺ and CD8⁺ T cells and B cells entered cell cycling faster than during the sensitization phase as typically observed for a secondary adoptive immune response (Fig. 2

a–f). Interestingly, NK cells also seemed to behave in an adoptive fashion with a stronger and faster response than observed for the sensitization phase peaking already at D2 (Fig. 2g and h). A twofold increase in the number of cycling NK cell in the elicitation phase compared with the sensitization phase was observed. Taken together, these experiments demonstrated that specific proliferating lymphocytes can be determined in the draining lymph nodes during the sensitization and elicitation phases of CHS. Furthermore, our results suggested that NK cells might be involved in CHS in an adoptive-like response, which is in agreement with a recent study (7).

Expression of T cell activations markers during the sensitization and elicitation phases of CHS

To further investigate T cell activation during the sensitization and elicitation phase of CHS, we next studied activations markers expressed at the cell surface of lymphocytes. We first tested down-regulation of CD62L (L-selectin) and up-regulation of CD44 on CD4⁺ and CD8⁺ T cells. CD62L is a lymph node homing receptor expressed at the surface on naïve T cells. CD62L is rapidly down regulated from the cell surface following T cell activation. CD44 is involved in cell–cell interactions, cell adhesion and migration, and is up-regulated at the T cell surface following T cell activation. Thus, CD44⁺CD62L[−] T cells represent recently activated T cell. Cells were isolated from the draining lymph nodes at the days indicated, stained with mAbs against CD4, CD8, CD62L, and CD44 and subsequently analysed by flow cytometry. During the sensitization phase, the occurrence of CD44⁺CD62L[−] T cells followed similar kinetics as the occurrence of BrdU⁺ T cells. CD44⁺CD62L[−] CD4⁺ T cells outnumbered CD44⁺CD62L[−] CD8⁺ T cells and their presence in the lymph node seemed to be more protracted compared with CD44⁺CD62L[−] CD8⁺ T cells (Fig. 3a–d). During the elicitation phase the occurrence of CD44⁺CD62L[−] T cells raised more rapidly as compared with the sensitization phase, supporting the adoptive nature of the response. Interestingly, in the elicitation phase CD44⁺CD62L[−] CD8⁺ T cells outnumbered CD44⁺CD62L[−] CD4⁺ T cells.

Next, we studied up-regulation of CD11a and CD25 on CD4⁺ and CD8⁺ T cells. CD11a constitutes the α -chain of the integrin receptor lymphocyte function associated antigen 1, which becomes up-regulated following T cell activation. CD25 is the α -chain of the interleukin-2 receptor that likewise becomes up-regulated following T cell

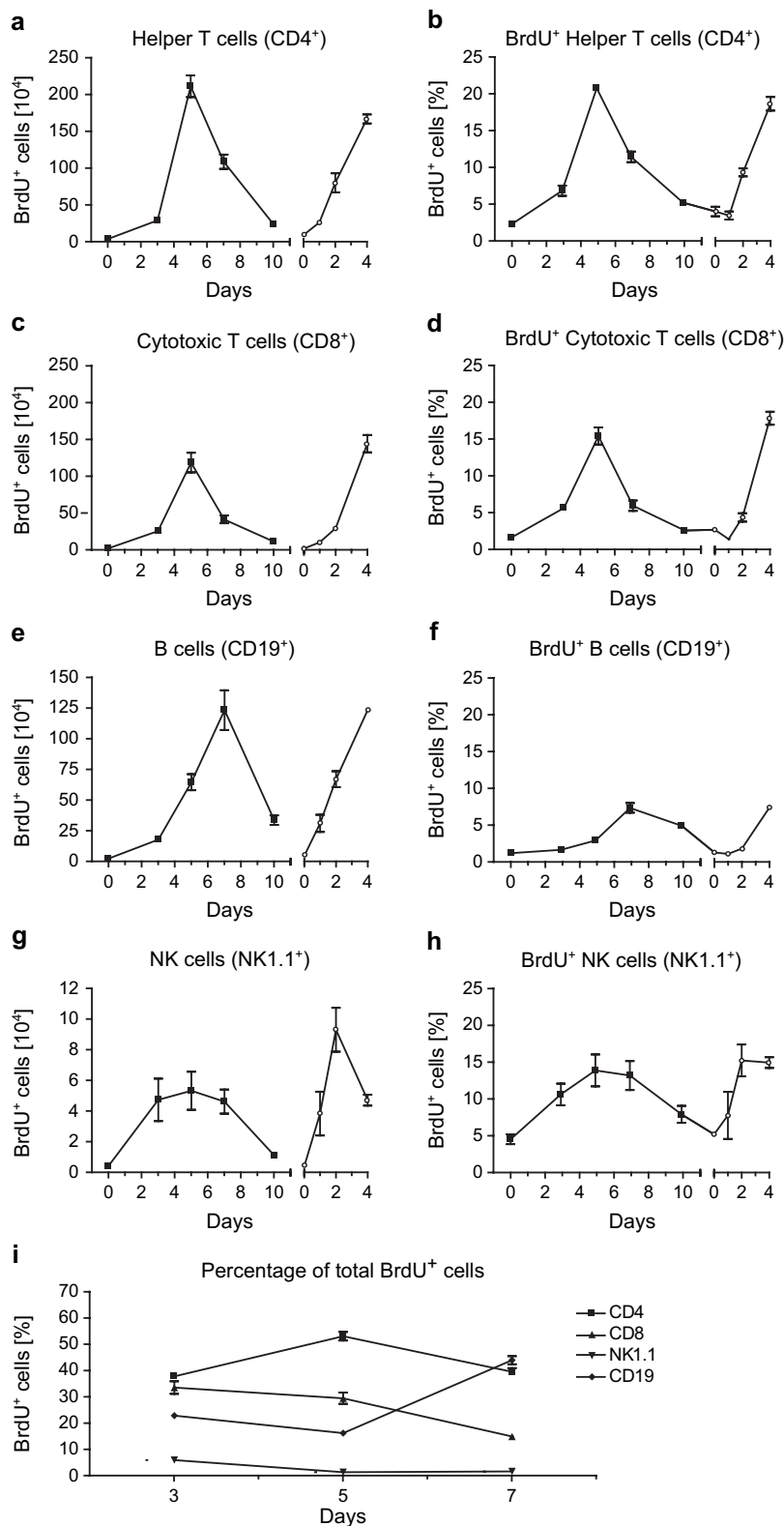


Fig. 2. Proliferative capacity of infiltrating cells during CHS. C57BL/6 mice received 25 μ l of 0.15% DNFB on the dorsum of both ears daily for 3 consecutive days. Groups of mice were euthanized on D3, D5, D7, and D10. Another group of mice was challenge with 0.15% DNFB and subsequently mice were euthanized on D1, D2, and D4. 2 days before mice were euthanized, they were given 0.8 mg/ml BrdU in the drinking water. Cell suspension from auricular lymph nodes were co-stained for BrdU incorporation and CD4 (a, b), CD8 (c, d), CD19 (e, f), and NK1.1 (g, h) expression and subsequently analysed by flow cytometry. (a, c, e, g) Shows the total amount of BrdU⁺ cells within the given cellular population. (b, d, f, h) Shows the percentage of BrdU⁺ cells within the given cellular population. ■ and ○ shows the kinetics of the sensitization phase and of the elicitation phase, respectively. (i) Shows the fractional contribution of each cellular population to the total amount of BrdU⁺ cells, in percentage. $n = 3-4$ mice per group from 2 independent experiments. CHS, contact hypersensitivity; DNFB, 2,4-dinitrofluorobenzene.

activation. During both the sensitization and elicitation phase of CHS, the occurrence of CD11a⁺CD25⁺ T cells followed the same kinetics as the occurrence of CD44⁺CD62L⁺ T cells, although the absolute number of CD11a⁺CD25⁺

T cells was slightly reduced compared with CD44⁺CD62L⁺ T cells (Fig. 3e–h). Again, CD11a⁺CD25⁺ CD8⁺ T cells outnumbered the CD11a⁺CD25⁺ CD4⁺ T cells in the elicitation phase.

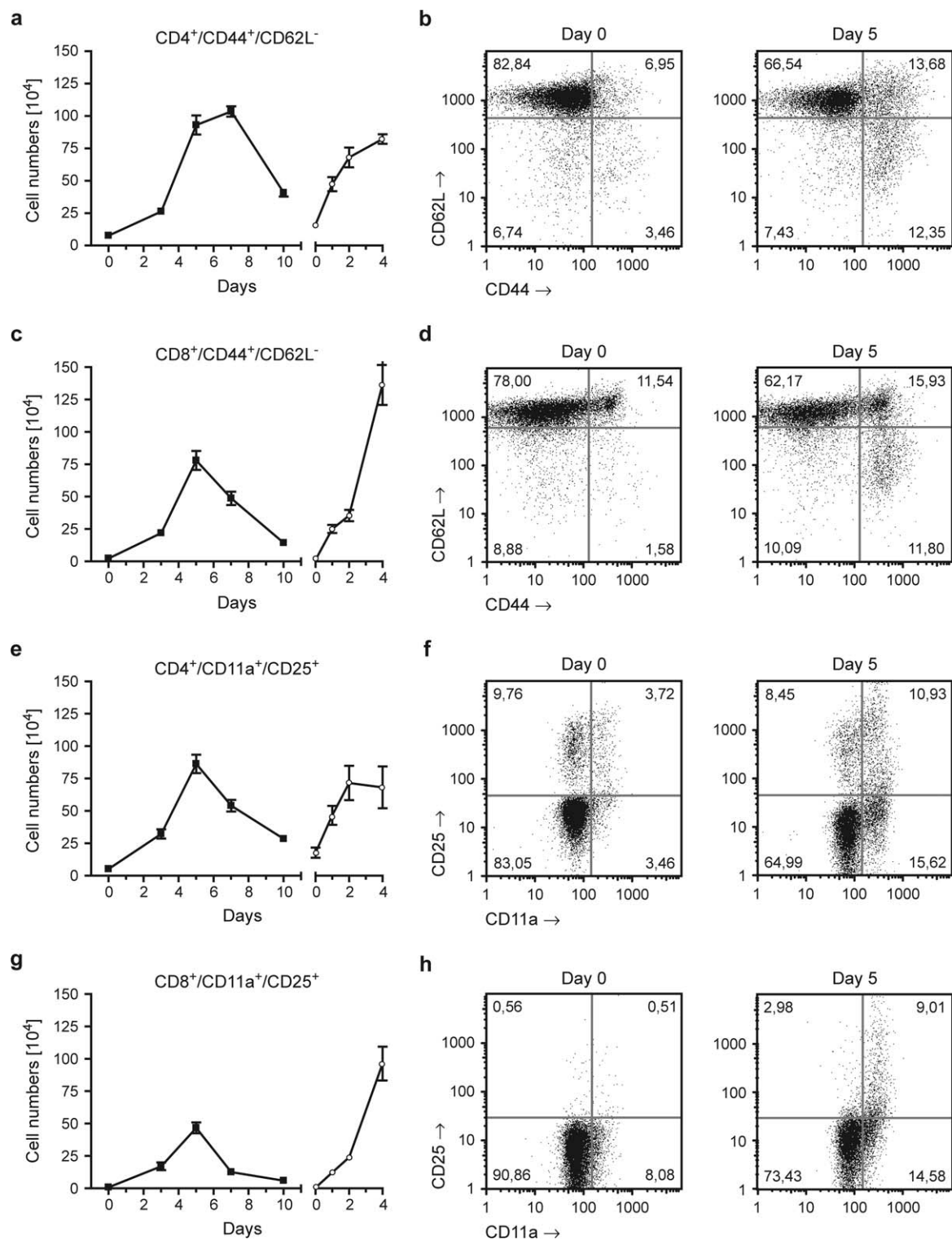


Fig. 3. The expression of T cell activation markers during CHS. C57BL/6 mice were painted with 0.15% DNFB for 3 consecutive days. On various days, draining auricular lymph nodes were collected and single-cell suspensions were made. The suspensions were co-stained for expression of CD4/CD62L/CD44, CD8/CD62L/CD44, CD4/CD11a/CD25, or CD8/CD11a/CD25, and subsequently cells were analysed by flow cytometry. In addition, groups of mice were challenge, and cell suspensions were made 1, 2, and 4 days post-challenge. (a, c, e, g) The kinetic for expression of the activated phenotypes during both sensitization and elicitation is shown as absolute numbers of cells with the activated phenotypes. Expression of CD44 versus CD62L on $CD4^+$ (b) and $CD8^+$ (d) T cells. The percentage of cells within each quadrant is indicated in the relevant quadrants. Expression of CD11a versus CD25 on $CD4^+$ (f) and $CD8^+$ (h) T cells. The percentage of cells within each quadrant is indicated in the relevant quadrants. ■ and ○ shows the kinetics of the sensitization phase and of the elicitation phase, respectively. CHS, contact hypersensitivity; DNFB, 2,4-dinitrofluorobenzene.

Taken together, these experiments demonstrated that expression of various activation markers of different cell populations in the draining lymph nodes can be precisely determined. Furthermore, our results indicate that whereas $CD4^+$ T cells seemed to be the dominating T cell subpopulation during sensitization, $CD8^+$ T cells seem to dominate during the elicitation phase.

Cell accumulation and cycling in the draining lymph nodes during CHS is antigen specific

To test whether the cell accumulation and activation we detected in the draining lymph nodes was antigen specific, we sensitized groups of mice with either DNFB or OXA. Following a rest period, each group was divided in 2; one was challenged with DNFB and the other with OXA. 1 day after challenge, mice were euthanized, and cell accumulation and proliferation were determined by the use of flow cytometry. Mice sensitized with DNFB demonstrated significant accumulation and proliferation of $CD4^+$ and $CD8^+$ T cells, B cells, and NK cells when challenged with DNFB but not with OXA (Fig. 4a and b). Likewise, mice sensitized with OXA demonstrated significant accumulation and proliferation of $CD4^+$ and $CD8^+$ T cells, B cells, and NK cells when challenged with OXA but not with DNFB (Fig. 4a and b). One might expect a sensitization response towards the challenge allergen in mice sensitized

with 1 allergen and challenge with another. However, in these experiments the challenge response was investigated already 1 day after only 1 allergen application, and we saw none or only a minor primary response at this time-point (Fig. 4 compared with Fig. 1). Taken together the method described in this paper can be used to test for accumulation and activation of antigen-specific cell populations in the draining lymph nodes during CHS.

Discussion

The purpose of the present study was to develop an improved method to investigate cell accumulation and activation in the draining lymph nodes during the sensitization and elicitation phase of CHS. We investigated cell infiltration, proliferation, and activation status of T, B, and NK cells using DNFB and OXA as allergens in a modified LLNA method applying multi-parameter flow cytometric analysis instead of traditional incorporation of radiolabelled thymidine. This method allowed us to determine the absolute number of any cell subpopulation in the draining lymph node, the fraction of these cells that have started cell cycling, and their activation status as determined by cell surface expression of activation markers.

It is known that contact allergens induce a number of changes within draining lymph nodes

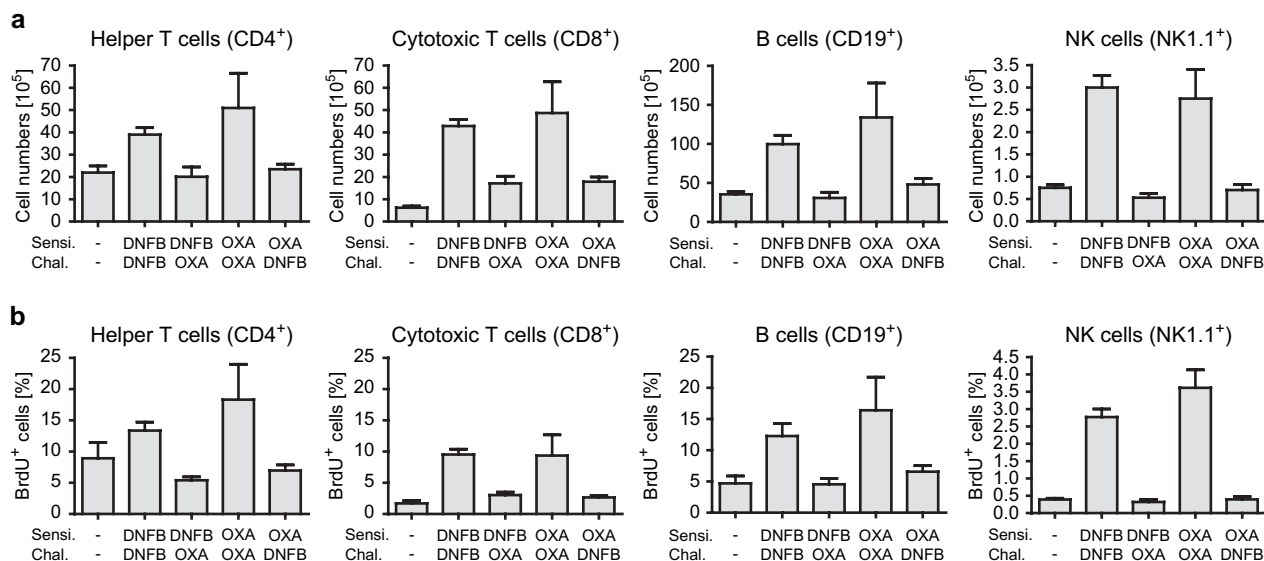


Fig. 4. CHS responses are hapten specific within the model. Mice were sensitized on 3 consecutive days by painting of 0.15% DNFB or 0.3% OXA on the dorsum side of both ears. 12 days later, both ears were challenged with 0.15% DNFB or 0.3% OXA. Mice were euthanized 2 days later and cell suspensions of the draining auricular lymph nodes were made. A group of untreated mice were included as a control. The cell suspensions were stained with anti-CD4, anti-CD8, anti-NK1.1, anti-CD19, and anti-BrdU and analysed by flow cytometry. Mice were sensitized with either DNFB or OXA and were challenged with either the same or the other hapten. (a) Cell infiltration, as absolute number of infiltrating cells. (b) Cell proliferation, as absolute numbers of $CD4^+$ T cells, $CD8^+$ T cells, NK1.1⁺ cells, and $CD19^+$ cells incorporating BrdU. $n = 3-4$ mice per group from 2 independent experiments. DNFB, 2,4-dinitrofluorobenzene; OXA, oxazolone.

including massive cell infiltration and accumulation. We found that $CD4^+$ and $CD8^+$ T cells are rapidly infiltrating the draining lymph node with a faster kinetics during elicitation than during sensitization. Approximately twice as many $CD4^+$ as $CD8^+$ T cells proliferated at D5 during sensitization as measured by BrdU incorporation. Likewise, approximately 50% more $CD4^+$ T cells compared with $CD8^+$ T cells expressed activation markers at D5 during the sensitization phase. These results indicate that $CD4^+$ T cells were the dominating cell type during sensitization. In contrast, during the elicitation phase equal numbers of $CD4^+$ and $CD8^+$ T cells became BrdU⁺, and a larger number of $CD8^+$ T cells expressed activation markers as compared with $CD4^+$ T cell, suggesting that $CD8^+$ T cells play a decisive role in the elicitation phase. This is in agreement with studies demonstrating that $CD8^+$ T cells is the main effector cells during the elicitation phase of CHS (1–3). However, we still found that as many $CD4^+$ as $CD8^+$ T cells were proliferating during the elicitation phase. A regulatory function has been suggested for $CD4^+$ T cells during CHS based on observations in knockout mice and studies using specific antibody-mediated depletion of $CD4^+$ T cells (1, 2). However, even though regulatory $CD4^+CD25^+FoxP3^+$ T cells have been shown to proliferated *in vivo* one cannot exclude a dual role of $CD4^+$ T cells in the CHS reaction ascribed to both effector and regulatory subsets (11). Indeed preliminary studies suggested that the $CD4^+$ T cell population where dominated by pro-inflammatory effector subsets as the number of $CD4^+FoxP3^+$ T cells did not exceed the number of proliferating $CD4^+$ T cells at the point of maximum inflammation in CHS sensitization and elicitation (data not shown). In support of this, a recent study indicates that Th17 cells, a newly found $CD4^+$ T cell subtype producing IL-17, play an important part of the inflammatory response in CHS (12).

Even though CHS is generally thought to be a T cell mediated immune response, a marked increase in B cell numbers within the draining lymph node has been reported (13–16). This is in accordance with our results, as we found that B cells were the dominating type of accumulating cell in the draining lymph node during the sensitization phase. B cells thus contributed to 50% of the total number of cells within the draining lymph node at D5 in the sensitization phase. In contrast, Suda et al. (16), showed that T cells, especially $CD4^+$ T cells, dominates the infiltration at this time-point in the sensitization phase. This difference might be explained by use of different mice strains, allergens, or experimental procedures. However, further experiments are

needed to clarify this discrepancy. Interestingly, even though we found that B cells are the dominating cell type in the draining lymph node during the sensitization phase of CHS, they only contributed to approximately 15% of the proliferating cells at D5, the day where lymph node proliferation is measured in the standard LLNA (9, 10). At D7, nearly half (48%) of the proliferating cells were B cells in agreement with the need of T cell help to get full activation of B cells. During the elicitation phase, nearly twice as many B cells were recruited to the draining lymph node as compared with the sensitization phase. Even though the number of B cells rapidly declined during the elicitation phase, they proliferated with the same kinetics as T cells and to nearly the same extend as the 2 T cell subpopulations.

Absence of B cells results in a reduced CHS response in various B cell deficient mice strains (4–6). Tsuji et al. (5, 6) have shown that B-1 cells, a subpopulation of B cells involved in innate immune responses, are involved in initiating CHS by providing IgM antibodies. The IgM antibodies form complexes with the allergen, and these complexes activate complement-generating C5a that leads to recruitment of allergen-specific effector T cells that mediate CHS responses. However, as B-1 cells are primarily found in the peritoneum and as they do not elicit memory responses, our results indicate that B-2 cells are involved in CHS. Thus, the role of B cell infiltration and proliferating within the draining lymph nodes during the phases of CHS needs to be further investigated.

A recent study have indicated that NK cells can induce CHS in T and B cell deficient mice, and that this response is adaptive (7). In accordance with this, we found infiltration and proliferation of NK cell during the sensitization and elicitation phases. As seen for T and B cells, both infiltration and proliferation of NK cell commenced at earlier time-point during the elicitation phase as compared with the sensitization phase. However, NK cells only contribute to 1–7% of infiltrating and proliferating cells during both phases of the response. Furthermore, we did not distinguish between NK cells and NK T cells in the present study, and can therefore not exclude that NK T cells are mediating the adoptive response. However, our results show that the reaction in the draining lymph node during the phases of CHS is a dynamic process involving various cell types belonging to both the innate and the adaptive immune system.

In conclusion, in the present study we introduce a method to simultaneously determine cellular infiltration, proliferation, and activation of various cell populations in the draining lymph nodes during the sensitization and elicitation phases of

CHS. The method is non-radioactive and easy to handle and will therefore be a good supplement to the standard LLNA. The method was allergen specific, as a response only was seen when the same allergen was used during sensitization and challenge (Fig. 4). The method might contribute to a better understanding of how specific allergens affect various cell populations during different phases of CHS and thereby contribute to the development of more efficient treatments of CHS.

Acknowledgements

This work was supported by the LEO Foundation. J. M. L. was recipient of a PhD scholarship from University of Copenhagen.

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