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Naive, Effector, and Memory T Lymphocytes Efficiently Scan Dendritic Cells In Vivo: Contact Frequency in T Cell Zones of Secondary Lymphoid Organs Does Not Depend on LFA-1 Expression and Facilitates Survival of Effector T Cells

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Naive, Effector, and Memory T Lymphocytes Efficiently Scan Dendritic Cells In Vivo: Contact Frequency in T Cell Zones of Secondary Lymphoid Organs Does Not Depend on LFA-1 Expression and Facilitates Survival of Effector T Cells[1]

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Contact between T cells and dendritic cells (DCs) is required for their subsequent interaction leading to the induction of adaptive immune responses. Quantitative data regarding the contact frequencies of T cell subsets in different lymphoid organs and species are lacking. Therefore, naive, effector, and memory CD4 T cells were injected into rats in absence of the cognate Ag, and 0.5–96 h later, spleen, lymph nodes, and Peyer's patches were removed. Cryosections were analyzed for contact between donor T cells and endogenous DCs in the T cell zone, and donor cell proliferation. More than 60% of injected naive CD4 T cells were in contact with endogenous DCs at all time points and in all organs analyzed. Surprisingly, we were unable to detect any differences between naive, effector, and memory CD4 T cells despite different expression levels of surface molecules. In addition, contact frequency was similar for T cells in lymphoid organs of rats, mice, and humans; it was unaffected by the absence of LFA-1 (CD11a/CD18), and sustained effector T cells in an activated state. Thus, the architecture of the T cell zone rather than expression patterns of surface molecules determines the contact efficiency between T cells and DCs in vivo. *The Journal of Immunology,* 2005, 174: 2517–2524.

T he interaction of T cells with dendritic cells (DCs)[3] is important for the function of the immune system (1, 2). Naive T cells are activated by interaction with mature DCs, which present the cognate Ag together with costimulatory molecules in the appropriate milieu of secondary lymphoid tissues (3). However, not only naive, but also effector and memory T cells interact with DCs (4). Effector T cells are able to kill DCs in secondary lymphoid organs, thereby contributing to the termination of an ongoing immune response (5). Furthermore, the decreased threshold of memory T cells to become activated compared with naive T cells (6) might be caused by higher contact frequency or longer contact duration with DCs. Naive, effector, and memory T cells differ considerably in the expression of adhesion molecules and chemokine receptors (7, 8). It is therefore reasonable to assume that each subset reveals a characteristic interaction pattern with DCs. To date, no such information is available. Thus, the aim of this study was to define differences in the T/DC interaction between naive T cells on the one hand, and effector and

memory T cells in contrast. For this reason, we have studied T cell zones of lymph nodes as well as that of spleen and Peyer's patches. The interaction of T cell subsets and DCs was analyzed in the absence of the cognate Ag. This allowed us to study the interaction of T cells with all DCs present in the tissues, rather than being limited to the few DCs carrying the cognate Ag. Using an established rat model (4), naive, effector, and memory CD4 T cells were injected, and their contact with endogenous DCs in the T cell zones of spleen, lymph nodes, and Peyer's patches was analyzed. In addition, LFA-1-deficient mice and human tonsils were used as a model system.

Approximately 60% of these injected naive CD4 T cells established contacts with endogenous DCs during migration through the T cell zone of lymphoid organs. Surprisingly, we were unable to detect any differences between naive, effector, and memory CD4 T cells. Moreover, the percentage of T cells in contact with DCs did not change over time; was comparable for spleen, lymph node, Peyer's patches, and tonsil; and did not differ between rat, mouse, and human lymphoid tissue. Furthermore, effector T cells in contact with DCs were maintained in cell cycle at a significantly higher proportion than those without contact.

Materials and Methods

Experimental design

To obtain naive and memory T cells, thoracic duct CD4 T cells from rats were separated into naive and memory phenotype, according to the high and low m.w. isoform of CD45R, respectively. Effector T cells were generated in vitro by stimulating rat lymph node lymphocytes via the TCR and CD28. T cells lacking LFA-1 expression were obtained from spleen and mesenteric lymph nodes of LFA-1-deficient mice. One type of donor T cell was injected, and the T cell zones of the host spleen, mesenteric lymph nodes, and Peyer's patches were examined at different time points.

Naive (CD45RC^{+}) and memory (CD45RC^{−}) CD4 T cells

Congenic rats from the inbred PVG.7A (RT7[a]) and PVG.7B (RT7[b]) strains were used. Details of the purification procedure were described previously

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[3] Abbreviations used in this paper: DC, dendritic cell; APAAP, alkaline phosphatase anti-alkaline phosphatase; CLSM, confocal laser scanning microscopy.

(8). Briefly, thoracic duct lymphocytes from PVG.7B donors were depleted of B cells, CD8$^+$ T cells, and CD90$^+$ recent thymic emigrants using a mixture of specific mouse mAbs and anti-mouse Ig-conjugated immunomagnetic particles. The resulting population contained >99% CD4$^+$ T cells; 80% of these were CD45RC$^+$. To obtain the CD45RC$^-$ population, the CD4$^+$ cells were additionally depleted of CD45RC$^+$ cells. A purity of >97% was routinely archived (9). It is known that memory cells (CD45RC$^-$) may revert back to the CD45RC$^+$ phenotype and re-express the high m.w. isoform of CD45R (10). Nevertheless, linking CD45RC$^+$ and CD45RC$^-$ with naive and memory is a useful division; it distinguishes T cells waiting to encounter Ag (naive) from those that have had experienced Ag (memory).

In vitro generated effector T cells

Rats from the standard inbred strain LEW/Ztm (RT.7^a) and the congenic strain LEW.7B/Won (RT.7^b) were used (11). Cell suspensions were prepared from peripheral lymph nodes (pooled axillary, brachial, and cervical lymph nodes) and mesenteric lymph nodes of LEW.7B rat. The cells were stimulated in vitro via the $\alpha\beta$ TCR (mAb R73) and CD28 (mAb JJ319) for 72 h, as described (11, 12). In average, 6×10^7 T cells were injected over 2 min i.v. into RT7^a Lewis rats. Because effector T cells generated from peripheral and mesenteric lymph nodes showed in the spleen the same interaction frequency with DCs and a comparable proliferation pattern, the data were pooled.

CD11a/CD18 (LFA-1)-deficient T cells

Spleen and lymph node cells from either LFA-1 (+/+) or (−/−) mice were pooled and labeled with digoxigenin, as described (13). Two hours and 24 h after i.v. injection of 4×10^7 cells, the spleen was removed, frozen in liquid nitrogen, and stored at −80°C. Two and 24 h after injection, >95% of the injected donor cells in the T cell zone were T cells.

Detection of donor cells and endogenous DCs in the recipient organs

At various times after injection, the rats were anesthetized with ether and exsanguinated. Within 5 min of cardiac arrest, spleen, mesenteric lymph nodes, and Peyer's patches were removed, frozen in liquid nitrogen, and stored at −80°C. Cryosections were air dried and stored at −20°C. To visualize in rat spleen, lymph node, and Peyer's patches the contact between the injected naive, effector, and memory CD4 T cells on the one hand and the endogenous DCs in contrast, two Ags were revealed simultaneously by immunohistochemistry, as described (11). In brief, the slides were fixed and incubated with mouse mAbs identifying either DCs (anti-CD54 (ICAM-1), 1A29; MHC class II, Ox6) or B cells (MARD-3 (anti-IgD); Serotec). Then the slides were incubated with the second Ab (rabbit anti-mouse; DakoCytomation) and the mouse Ab complex (alkaline phosphatase anti-alkaline phosphatase (APAAP); DakoCytomation). To identify the congenic cells, the biotinylated Ab His41 (RT7^b, anti-CD45.2) was used (30 min, dissolved in 20% mouse serum; Dianova (14)), followed by avidin-peroxidase (Dianova). The donor cells were revealed using diaminobenzidine (brown), and DCs or B cells were visualized using fast blue. With the same technique, the contact between endogenous DCs and endogenous T cell subsets was analyzed ($\alpha\beta$ T cells, mAb R73; Vβ8.2, mAb R78; Vβ10, mAb G101).

To visualize the contact between donor cells and DCs in mice, cryosections of the spleen were processed, as described above. DCs were first identified by CD205 (DEC-205) expression (rat mAb NLDC 145) and the APAAP technique (blue). The donor cells were then identified using a peroxidase-conjugated anti-digoxigenin Ab (brown).

Detection of donor T cells and endogenous DCs using confocal laser scanning microscopy (CLSM)

Tissue processing was performed, as described above, except fluorescence-conjugated Abs were used. Fluorescence microscopy was performed using a Leica TSC NT confocal microscope (Leica Microsystems) equipped with a ×100/1.4 lens and an argon-krypton mixed gas laser emitting blue (488 nm) and green light (568 nm). Serial confocal optical sections, each ~0.6 μm in thickness, were recorded for the selected regions, so that complete T cells and DCs were included in the stacks. The two fluorescences were regarded as colocalized (membrane contact) when yellow pixels, representing a volume of 0.3 μm × 0.3 μm × 0.6 μm, were detected. Maximum projections were calculated from the image stacks providing an extended depth of focus.

Detection of endogenous T cells and DCs in human tonsils using CLSM

Tissue samples of normal human tonsils, resected during routine tonsillectomies, were frozen in liquid nitrogen and stored at −80°C. The local ethical committee of Hannover Medical School approved the use of human tonsil tissue. Cryosections, 15 μm in thickness, were fixed in methanol-acetone (1:1) at −20°C for 10 min, rehydrated in PBS, and incubated in a mixture of a mouse anti-human CD3 Ab (clone Hit3a, isotype IgG2a) and a mouse anti-human CD11c Ab (clone KB90, isotype IgG1). After rinsing in PBS, the primary Abs were detected by a mixture of anti-IgG2a and anti-IgG1 isotype-specific secondary Abs conjugated to fluorescein and rhodamine, respectively (Southern Biotechnology Associates). CLSM was performed, as stated above.

Proliferation of effector T cells in vivo

To study the local proliferation of effector T cells in the T cell zone of the spleen, congenic donor cells were activated and injected, as described (11). Three days later, the rats received 5 mg of BrdU/100 g body weight i.v., and 1 h later the spleen was removed. Thus, only those lymphocytes that were in the S phase of the cell cycle within the respective microenvironment were labeled. As described above, injected cells (blue) and the endogenous DCs (brown) were first identified by using mAb directed against the congenic phenotype (CD45.2, His41) and CD54 (1A29), respectively. Next, incorporated BrdU in activated lymphocytes was identified, as described (15). To identify effector cells in all phases of cell cycle, splenic tissue was stained for the rat homologue of the Ki-67 Ag, as described (16).

Evaluation

The sections were evaluated using a light microscope using an ocular grid divided into 100 squares. In each tissue, >200 T cells were analyzed for

FIGURE 1. The interaction of congenic CD4 T cells and endogenous DCs in the rat spleen is shown 1 day after injection. *A*, Cryosection stained for injected CD4 T cells (brown, mAb His41 CD45.2; peroxidase technique) and CD54 expression (blue, mAb 1A29; APAAP technique) delineating the different compartments of the spleen (pals, periarteriolar lymphocytic sheath = T cell zone; ca, central artery; fo, follicle; mz, marginal zone; rp, red pulp; counterstain hematoxylin; magnification ×100). *B*, Higher magnification of an area of the pals indicated in *A*. Shown are CD4 T cells that are in contact with DCs (filled arrows) or not (open arrow; magnification ×1000). *C* and *D*, Identical with *A* and *B* with the exception that DCs are detected by staining for MHC class II expression (blue, mAb Ox6).
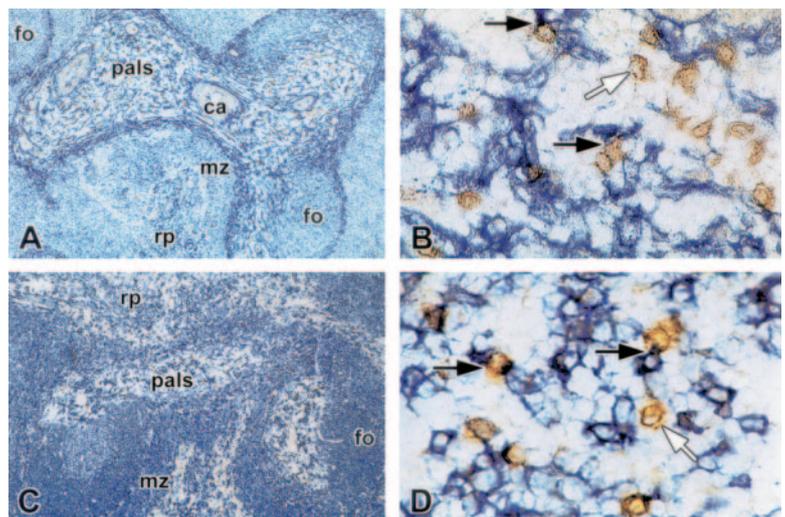
contact to DCs. At least three different sections per tissue were scored, thereby evaluating more than two different T cell zones. Means ± SD were determined using SPSS for Windows. Differences were analyzed using the Wilcoxon matched-pairs signed-ranks test ($p < 0.05$ was considered significant).

Results

The majority of injected CD4 T cells are in contact with DCs in the T cell zone of lymphoid organs

One day after injection of thoracic duct CD4 T cells, these cells could clearly be located in the T cell zone of rat lymphoid organs (brown; Fig. 1). The endogenous DCs in the T cell zone were identified by their strong staining for CD54 and MHC class II, and their typical morphology (Fig. 1). In such preparations, it was possible to define whether T cells were in contact with DCs (filled arrows; Fig. 1) or not (open arrows). Surprisingly, 1 day after injection, the most CD4 T cells were in contact with DCs (Fig. 2). The percentage was similar for spleen, lymph node, and Peyer's patches, and did not differ based on whether CD54 or MHC class II staining was used to identify the DCs in the T cell zone.

Adhesion molecules and chemokines do not modulate the frequency of contacts between CD4 T cells and DCs

Naive, effector, and memory CD4 T cells differ considerably in the expression of adhesion molecules and chemokine receptors, suggesting that they form different frequencies of contacts with DCs. To test this hypothesis in vivo, the subpopulations were injected, and the frequency of contacts between T cells and DCs was analyzed 1 day later. Surprisingly, naive, effector, and memory CD4 T cells did not differ, but showed a comparable contact frequency with DCs in spleen, lymph node, and Peyer's patches (Fig. 3). A kinetic analysis in the T cell zone of the spleen demonstrated that most CD4 T cells were in contact with DCs, and that this number did not change from 0.5 to 96 h after injection (Fig. 4). Together, these data show that the contact between CD4 T cells and DCs in the T cell zone of lymphoid organs is not influenced by the expression of surface molecules that are differently expressed on naive, effector, and memory CD4 T cells. To confirm this notion, LFA-1-deficient mice were used. The binding of CD11a/CD18
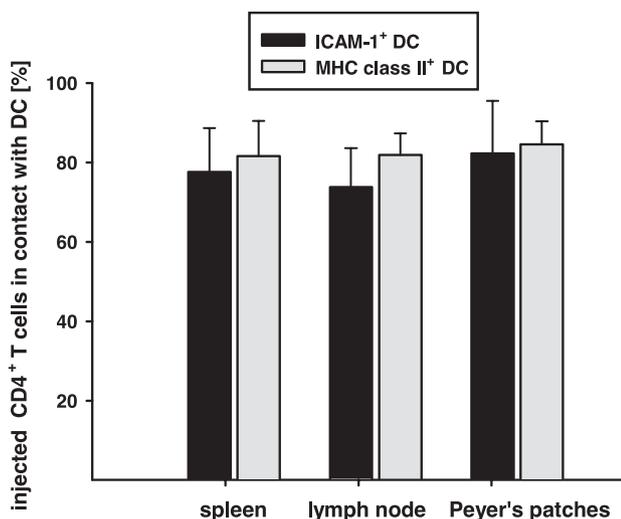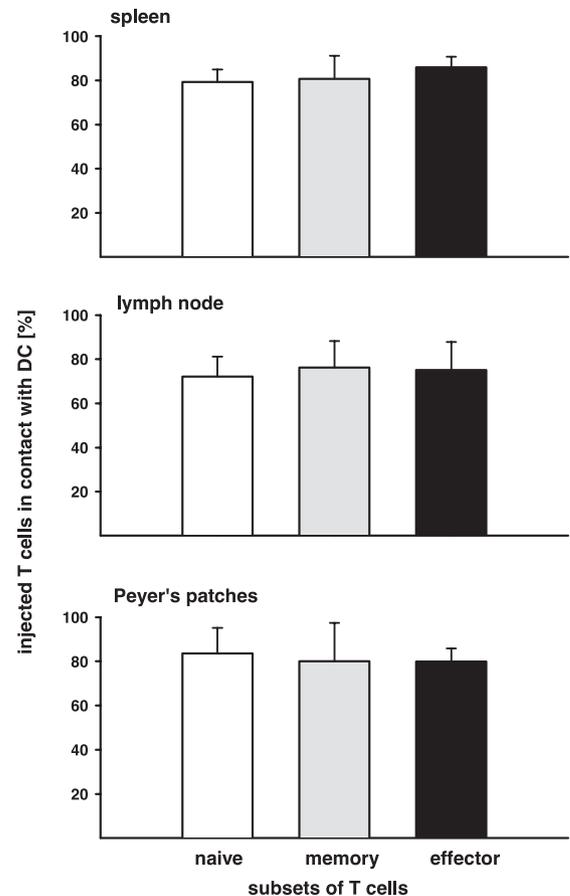

FIGURE 3. Naive, effector, and memory CD4 T cells do not differ in their contact frequencies with DCs in the T cell zone of lymphoid organs. One day after injection, congenic CD4 T cell subsets were localized in the T cell zone of spleen, lymph node, and Peyer's patches (mAb His41). The percentage of T cells in contact with DCs, identified by their CD54 expression (mAb 1A29), is indicated. Values represent means ± SDs (number of animals analyzed, $n = 5$–7).

(LFA-1) on the T cell surface to CD54 on the DC surface is important for T/DC interaction (17). Thus, wild-type and LFA-1-deficient cells were injected into wild-type mice, and the contact
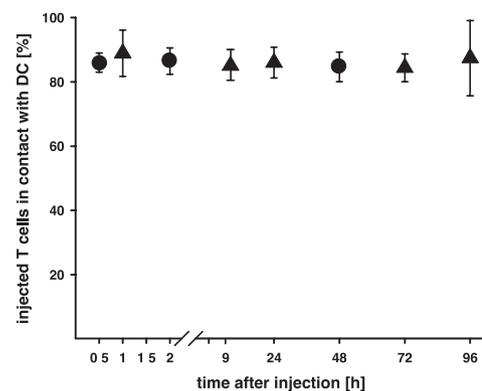

FIGURE 2. In lymphoid organs, the majority of CD4 T cells are in contact with DCs. One day after injection, congenic CD4 T cells were localized in the T cell zone of spleen, lymph node, and Peyer's patches. The percentage of T cells in contact with DCs, identified by their CD54 and MHC class II expression, is indicated. Values represent means ± SDs (number of animals analyzed, $n = 6$).

FIGURE 4. The contact frequencies between CD4 T cells and DCs do not change over time. From 0.5 to 96 h after injection, congenic CD4 T cells were localized in the T cell zone of the spleen (CD45.2, mAb His41). The percentage of T cells in contact with DCs identified by their CD54 expression (mAb 1A29) is indicated. Values represent means ± SDs (number of animals analyzed, $n = 3$–7; circles, naive and memory CD4 T cells; triangles, effector T cells).
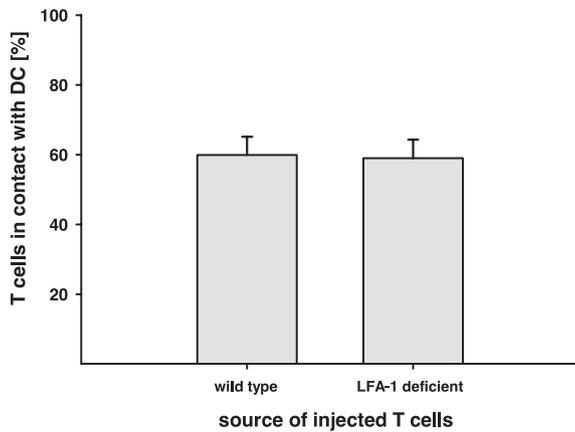
FIGURE 5. LFA-1 does not influence the contact frequencies between T cells and DCs in vivo. Two hours after injection of wild-type T cells or T cells from LFA-1-deficient mice, cells were localized in the T cell zone of the spleen. The percentage of T cells in contact with DCs, identified by their CD205 expression (mAb NLDC 145), is indicated. Values represent means $\pm$ SDs (number of animals analyzed, $n = 6$).

frequencies within the T cell zone were determined 2 h after the injection. Supporting the rat data, in mice there was no difference between wild-type and LFA-1-deficient T cells (Fig. 5). Even

though we did not separate the cells before injection, after 2 and 24 h, >95% of the injected cells in the T cell zones were T cells. In addition, a comparable distribution of the two T cell populations (wild type and LFA-1 deficient) was found 24 h after injection (data not shown).

When analyzing the contacts between T cells and DCs in conventional microscopy, it is not possible to assess whether the membranes of the two cell types are so closely approximated that they could functionally interact via surface molecules. Therefore, CLSM on thick cryosections containing complete either naive or memory T cells was performed (Fig. 6A). Simultaneously, DCs were stained (Fig. 6B), and in the maximum projection (Fig. 6C) the T cells could be identified as having contact with DCs (asterisk) or as not having contact (triangle). Subsequently, T cells in contact with DCs were scanned from the upper to the lower section surface by 0.6-μm-thick optical sections, and the proximity of T cell and DC membranes was recorded (arrow; Fig. 6, D–L). The analysis shows that the cell membranes of the T cells and DCs indeed were in close contact. Interestingly, once T cells were in contact with DCs, almost 40% of these T cells had a contact zone large enough to be detected in each optical section analyzed (33 of 84 cells analyzed).

Analyses by CLSM revealed that 60% $\pm$ 5 (mean $\pm$ SD of 8 mice) of both naive and memory T cells were in contact with DCs. This value is lower than that obtained in conventional microscopy.
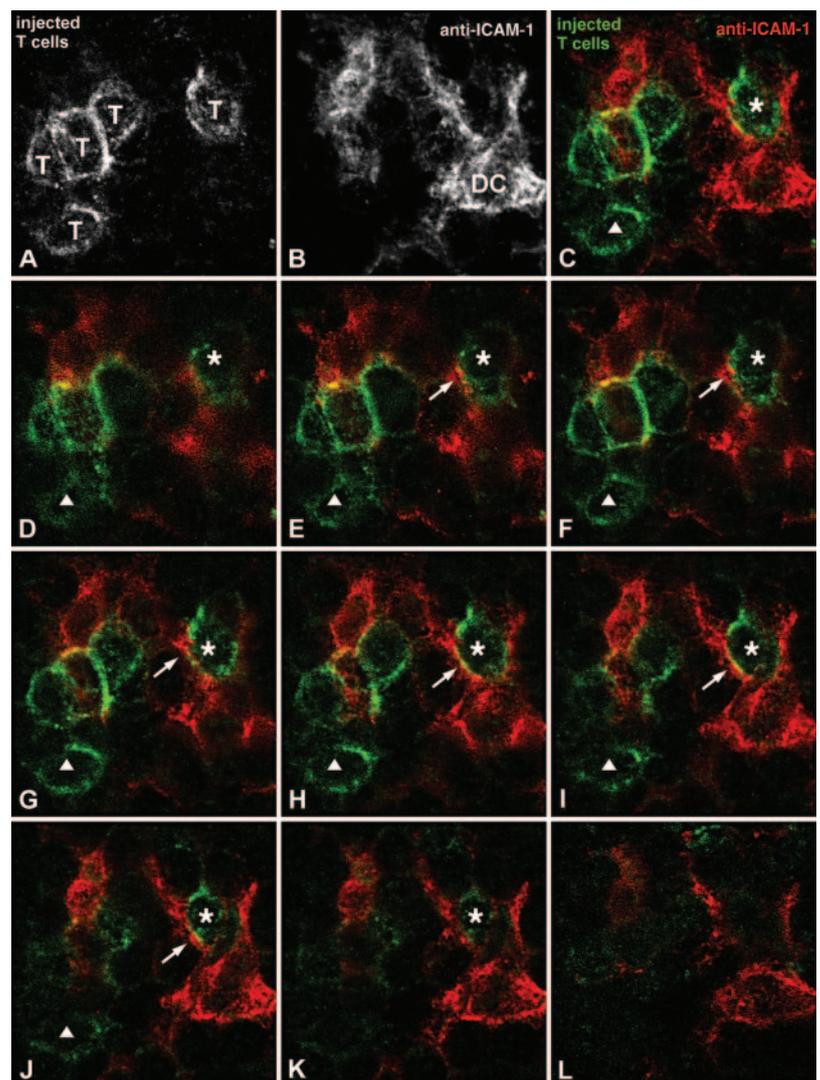
FIGURE 6. CLSM showing naive CD4 T cells and DCs in the T cell zone of the spleen 1 day after injection of congenic T cells. *A*, Maximum projection of injected T cells recorded in green channel (CD45.2, mAb His41). *B*, Maximum projection of endogenous DCs identified by their CD54 expression and recorded in red channel (mAb 1A29). *C*, Overlay image of *A* and *B*. The asterisk marks a T cell in contact with a DC, whereas the triangle denotes a T cell having no contact. *D–L*, Single optical section (thickness 0.6 μm) of a confocal image stack that contains T cells with (asterisk) and without (triangle) contact with DCs. The arrows indicate areas in which the cell membranes of T cell and DC are closely approximated (green + red = yellow). A total of 140 T cells in 8 animals was studied, and 9.5 $\pm$ 1.9 optical sections per T cell were analyzed (magnification $\times$1000).

Table I. *The majority of T cells without contact with DCs are located in close vicinity to DCs*

Distance from DC (μm)	Number of Injected T Cells (%)[a]
<5	56 ± 7
5–10	26 ± 7
10–15	13 ± 3
>15	5 ± 2

[a] CD4 T cells were injected, and 1 day later the number of cells with and without contact to DCs was identified, as described. Subsequently, CD4 cells without contact to DCs were analyzed regarding their distance away from adjacent DC. In each animal, ~50 CD4 T cells without DC contact were scored into the four categories, and the respective percentage was indicated. Values are means and SDs ($n = 7$). DCs were identified by their typical morphology and CD54 expression.

Identification of injected T cells and DCs using enzymatic color reaction leads to a broader labeling pattern. Compared with CLSM, this increases the number of T cells in contact with DCs, and implies that T cells scored as having no contact are closely located to the next DC. Indeed, analyses of injected T cells without contact to DCs in two-dimensional sections revealed that most of these cells were <5 μm apart from the next DC (Table I).

Thus, CLSM shows that within the T cell zones of secondary lymphoid organs most T cells establish contact with DCs, allowing functional interactions, and it confirms the finding of the conventional microscopy that there is no difference between naive and memory T cells.

The high contact frequency of T cells and DCs is seen in various species and functional conditions

To test whether the observed contact frequencies also apply to endogenous T cells, various populations of endogenous T cell population were stained, and their contact with endogenous DCs was determined. As summarized in Table II, the contact frequencies of endogenous T cells with DCs are similar to that of the injected naive, effector, and memory CD4 T cells. Furthermore, the same interaction frequencies were found in the T cell zones of mice spleen and human tonsils (Table II). Thus, the almost identical values obtained in different species and populations of T cells indicate that the high contact frequency between T cells and DCs is a characteristic feature of the T cell zone of secondary lymphoid organs. Additionally, neither an immune response in the spleen induced by the injection of SRBC nor denervation of the spleen affected the contact frequency between T cells and DCs (Table II), making this a stable feature over a broad range of cells and conditions of the immune system.

Effector T cells in contact with DCs maintain proliferation at a higher level

To investigate whether contact with DCs in the T cell zone of the spleen maintains them in the S phase, effector T cells were injected i.v. One hour before the spleen was removed, the animals received the nucleotide-analog BrdU that labels cells in the S phase of the cell cycle. This allowed identification of: 1) injected effector T cells (blue; Fig. 7), 2) endogenous DCs (brown), and 3) BrdU (red). In addition, effector T cells could be classified as having contact with DCs and as having incorporated BrdU (filled arrow, Fig. 7) or not (open arrows). Using a comparable approach, effector T cells, DCs, and the Ki-67 Ag were stained to identify all cells in the cell cycle. Both 24 and 72 h after injection, a considerable fraction of the effector T cells remained in the cell cycle and had entered S phase (Fig. 8). Surprisingly, contact with DCs was correlated with a significant increase in the number of effector T cells both in cell cycle and in S phase of the cell cycle. The analysis was extended to determine whether the site of localization of the contact between effector T cells and DCs within the T cell zone was of importance. The frequency of effector T cells in the cell cycle was the same regardless of whether contact with DCs took place in the inner or outer region of the T cell zone (Fig. 9). However, the number of effector T cells entering the S phase was significantly higher when the contact with DCs occurred in the outer region of the T cell zone, an area in which a majority of effector T cells had an additional contact with B cells (Fig. 9).

Discussion

DCs make up <1% of the cells in lymphoid organs (18, 19), leading to the notion that they are a spare population (20). Consequently, molecular mechanisms seem necessary, which enables T cells to meet rare DCs in vivo at all (e.g., expression of chemokine receptors on T cells and release of chemokines by DCs). However, the present study shows that after injection of CD4 T cells, >60% of these cells are in contact with endogenous DCs in the T cell zone of secondary lymphoid organs at all time points investigated, which is in agreement with an observation in mice mentioned in passing (21). Moreover, this high value even underestimates the real number of T cells in contact with DCs in vivo. It has recently been shown by analyzing the interaction of T cells with single DCs, using two-photon microscopy, that T cells also establish contacts with the thin dendrites of DCs (22), some of which could have been missed by the techniques applied in the present study. In addition, we show that most T cells, which are not in contact with DCs, are <5 μm apart from the next one. Thus, because of the high velocity of 11 μm min^{-1} of the T cell movement in lymphoid

Table II. *Percentage of T cells with contact to endogenous DCs in the T cell zone of secondary lymphoid tissue of various species*

Subset of Cells	Contact to DCs (%)[a]	Tissue	Species
αβ T cells	62 ± 2 ($n = 5$)	Normal spleen	Rat
Vβ8.2 T cells	67 ± 4 ($n = 5$)	Normal spleen	Rat
Vβ10 T cells	64 ± 2 ($n = 5$)	Normal spleen	Rat
All cells in T cell zone	66 ± 4 ($n = 9$)	Normal spleen	Rat
All cells in T cell zone	56 ± 3 ($n = 6$)	Normal spleen	Mouse
CD3	68 ± 4 ($n = 7$)	Tonsil	Human
αβ T cells	65 ± 4 ($n = 6$)	Spleen challenged with SRBC[b]	Rat
αβ T cells	64 ± 2 ($n = 6$)	Spleen without innervation[c]	Rat

[a] Values are means and SDs (in brackets the number of animals analyzed). DCs were identified by the expression of CD54 in rats, CD205 in mice, and CD11c in humans.
[b] Rat spleens were challenged by i.v. injection of 1×10^{10} SRBCs, and 10 days later, at the height of germinal center reaction, the frequency of contacts between T cells and DCs was determined.
[c] The innervation of rat spleens was surgically removed, as previously described (24), and 10 days later, when the epinephrine content in the spleen was reduced below 10% of its normal value, the frequency of contacts between T cells and DCs was analyzed. Contact between T cells and DCs was identified either with CLSM or conventional enzyme immunohistochemistry.
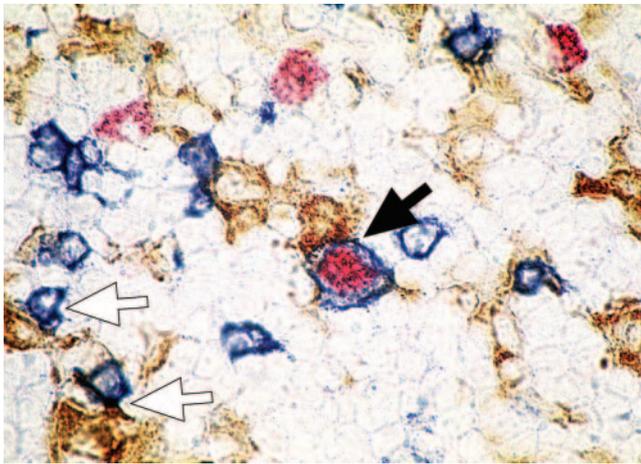
FIGURE 7. Interaction of congenic effector T cells in the S phase of the cell cycle and endogenous DCs is shown in the rat spleen. Three days after injection of in vitro activated effector T cells, the animals received BrdU i.v., and 1 h later the spleen was removed. Cryosections were stained for injected effector T cells (blue, CD45.2, mAb His41; AP technique), CD54 to identify DCs in the T cell zone (brown, mAb 1A29; peroxidase technique), and incorporated BrdU (red; APAAP technique). Contacts of effector T cells with DCs are indicated (filled arrow, effector T cell-incorporated BrdU; open arrows, effector T cells that did not incorporate BrdU; magnification ×1200).

tissues (23–25), even the T cells not in contact with DCs at the time of analysis will be in contact in less than 1 min.

Because naive, effector, and memory CD4 T cells differ considerably in the expression of adhesion molecules and chemokine receptors, which in turn are important in mediating T/DC contacts, we expected to find differences in the contact frequency between the various T cell subsets and DCs. Surprisingly, the current investigation shows that the high contact frequency is similar whether we looked at all T cells or the injected subpopulations of naive, effector, and memory CD4 T cells. Because the frequencies of all T cells and that of CD4 T cells are comparable, it is very likely that the CD8 T cells have identical contact frequencies too. Even small endogenous T cell populations such as Vβ8.2 and Vβ10 T cells (~1% of $\alpha\beta$ T cells) reveal contact frequencies within the same range. In addition, despite the fact that spleen, lymph nodes, and Peyer's patches contain different ratio of mature and immature DCs (26) and DC subpopulations (27, 28), the contact frequency between T cell and DCs is comparable. Because the presentation of cognate Ag increases the time that naive T cells interact with DCs (29, 30), it will be important to determine whether and how T cell subsets differ in this regard. Thus, the present study shows that irrespective of surface molecule expression, including CD11a/CD18 (LFA-1), lymphoid organ, and species analyzed, the number of T cells in contact with DCs in the T cell zone of lymphoid organs is within the same range.

FIGURE 8. Effector T cells are maintained in the cell cycle at a higher rate when they are in contact with DCs. One and 3 days after injection, congenic effector T cells were divided in two groups, depending on whether they have no contact (○) or have contact (●) with DCs. Then the percentages of effector T cells in the cell cycle (*left side*; Ki-67 positive) and in the S phase of the cell cycle (*right side*; BrdU positive) are indicated. Values belonging to the same spleen are connected. Means ± SDs are given. The asterisks indicate significant differences between effector T cells without and with contact to DCs ($p < 0.05$; Wilcoxon matched-pairs signed-ranks test).
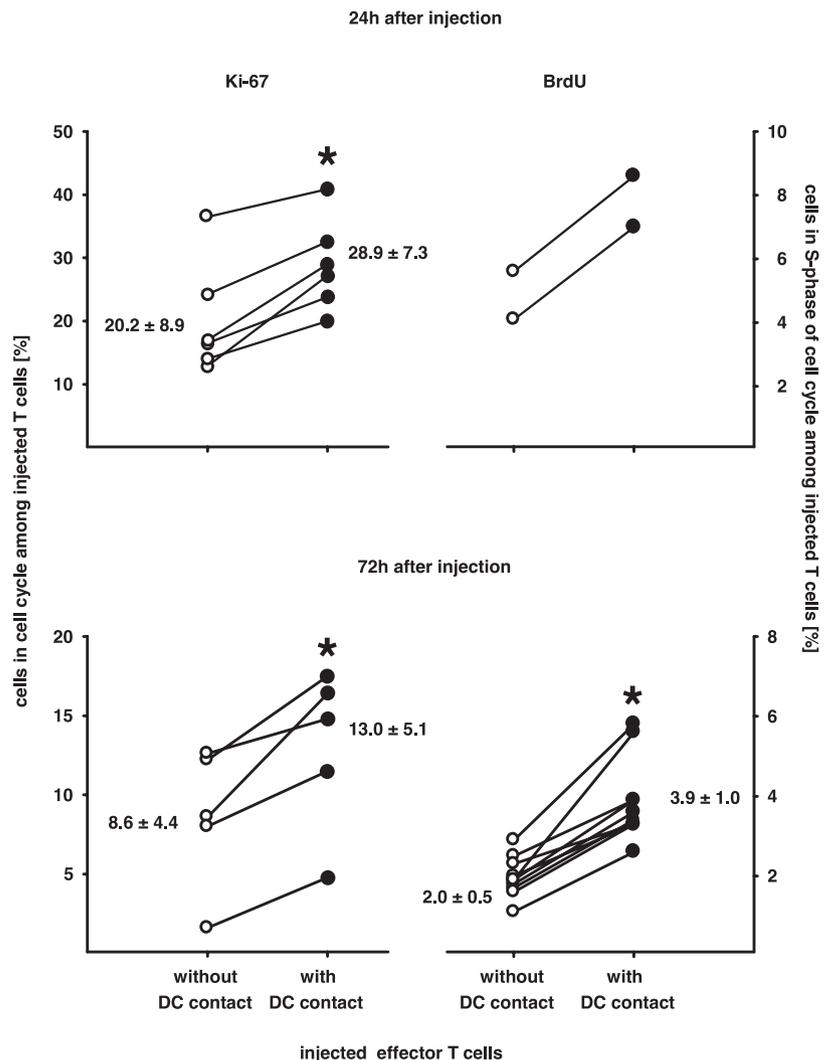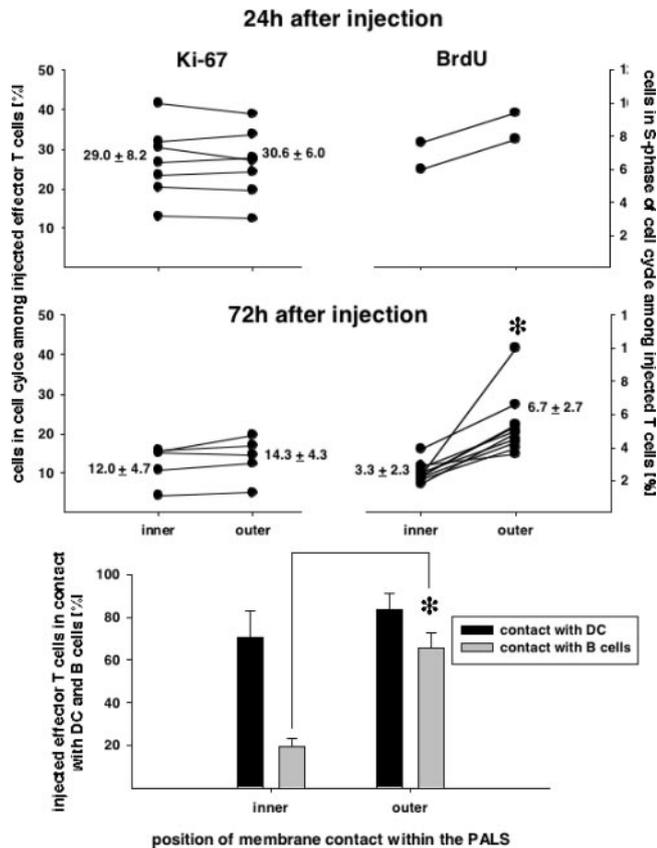
FIGURE 9. Effector T cells in S phase of the cell cycle and in contact with DCs are more prevalent in the outer regions of the T cell zone. The T cell zone was subdivided into two regions, inner (>20 μm distant from marginal zone or follicle) and outer (<20 μm), and congenic effector T cells in contact with DCs were recorded 1 day (*upper panel*) and 3 days (*middle panel*) after transfer. The percentages of effector T cells in cell cycle (*left side*; Ki-67 positive) and in the S phase of the cell cycle (*right side*; BrdU positive) are indicated. Points connected by lines are from the same spleen. Mean values ± SDs of effector T cells in the inner and outer regions are compared (Wilcoxon matched-pairs signed-ranks test) for significant differences (∗, $p < 0.05$). *Lower panel*, The percentage of congenic effector T cells being in contact with DCs (CD54, mAb 1A29) and B cells (IgD, mAb MARD) is indicated either in the inner or the outer part of the T cell zone. Values represent means ± SDs (number of animals analyzed, $n = 5$).

Although the entry of T cells and DCs into the T cell zone of secondary lymphoid organs is regulated by molecules such as the chemokine receptor CD197 (CCR7) (31) and adhesion molecules (32), differentially expressed surface molecules are probably not involved in establishing the initial contact between T cells and DCs within the T cell zone. This conclusion is supported by the observation that in vivo T cells migrate randomly through the T cell zones and not along chemokine gradients (22, 33). In contrast to T cells, most B cells leave the T cell zone and enter the B cell zone (34, 35). If this process is regulated by a chemokine gradient, directed movement of B cells in the T cell zone should be observed. Thus, the anatomy of the DC network and the architecture of the T cell zone appear to be major factors determining the interaction of T cells and DCs in vivo. Adhesion molecules and chemokine receptors are probably more important in influencing the functional outcome of those interactions, e.g., they determine whether a cellular (Th1-like) or humoral (Th2-like) immune response will be induced.

The high frequency of T cells in contact with DCs suggests that this interaction could be linked to mechanisms that maintain the size of the peripheral T cell pool and help to keep up peripheral tolerance (36, 37). It is known that naive CD4 T cells need contact with MHC class II-expressing cells, presenting mainly self peptides, to survive and to become preactivated (38). Our data indicate that DCs in the T cell zone could serve this function. For example, 1 day after cell transfer, ~20% of effector T cells in the spleen without contact with DCs are in the cell cycle, whereas 30% are in cell cycle when they are in contact with DCs. Furthermore, contact to DCs not only increases the number of effector T cells in cell cycle, but also increases the number of effector T cells in the S phase of the cell cycle in the T cell zone of both the spleen (present study) and the lymph node (11). Interestingly, although there is no difference in the overall distribution of effector T cells in the cell cycle, significantly more effector T cells are in the S phase when the contact to DCs occurs in the outer part of the T cell zone. At this location, most of them have additional contact to B cells, which may be needed to push them into the S phase. Thus, the combination of randomly moving effector T cells and the characteristic architecture of the T cell zone (harboring mostly B cells in the outer part) may provide the explanation for the observation that after challenge T cells in S phase accumulate in the outer part of the T cell zone (summarized in Ref. 39).

More than 60% of the T cells contained within the human body reside in the T cell zone of secondary lymphoid organs (40). In the species analyzed (human, rat, mouse), the architecture of the T cell zone itself, with its extensive network of DCs, facilitates the high frequency of contacts between T cells and DCs. This shows that it is the rule, rather than the exception, that T cells are in contact with DCs in vivo.

Because T cells need ~24 h to pass through lymph nodes (41), and because during this time >60% have contact with DCs, T cells have a cumulative contact time with DCs of >12 h (60% of 24 h) during each lymph node passage. According to a recent publication by Miller et.al (22), the contact between a DC and a T cell lasts ~3 min. Therefore, in these 12 h, a single T cell could potentially contact ~240 DC. Thus, in vivo the contact of T cells with DCs per se is not the limiting factor for induction of an adaptive immune response. Instead, the position of DCs within the T cell zone might be of crucial importance (42). If clustered at one site, DCs are probably much more effective to activate randomly moving T cells (24, 25, 29, 33) than the same number of Ag-presenting DCs distributed all over the T cell zone. Supporting or preventing the formation of such regions might be a way of inducing helpful and preventing harmful immune responses, respectively.

Acknowledgments

Disclosures

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