The Journal of Immunology

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J Immunol 2002; 169:638-641; ;
doi: 10.4049/jimmunol.169.2.638
http://www.jimmunol.org/content/169/2/638

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

CUTTING EDGE

Cutting Edge: CCR7$^+$ and CCR7$^-$ Memory T Cells Do Not Differ in Immediate Effector Cell Function[1]

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It has been proposed that expression of the chemokine receptor CCR7 represents a defining factor for nonpolarized central (CCR7$^+$) and polarized effector memory (CCR7$^-$) T cells. In this study, we have tested this hypothesis using in vivo-activated T cells from P14 and SMARTA TCR-transgenic (tg) mice specific for MHC class I- and II-restricted epitopes of the lymphocytic choriomeningitis virus (LCMV) glycoprotein. CCR7 cell surface expression on TCR-tg cells was monitored with a CC chemokine ligand 19-Ig fusion protein. CC chemokine ligand 19-Ig staining separated TCR-tg cells activated by LCMV infection into CCR7$^-$ and CCR7$^+$ effector/memory T cell populations. Nonetheless, both T cell populations isolated from spleen and liver produced identical amounts of IFN-γ after short-term Ag stimulation. Furthermore, CCR7$^+$ and CCR7$^-$ CD8 TCR-tg cells from LCMV-infected mice exhibited similar lytic activity against LCMV peptide-coated target cells. These results question the proposed concept of differential effector cell function of CCR7$^+$ and CCR7$^-$ memory T cells. ***The Journal of Immunology*****, 2002, 169: 638–641.**

Chemokine receptors are known to be differently expressed on naive and activated T cells. Naive T cells express CCR7, a receptor for the constitutive chemokines, CC chemokine ligand (CCL)[3] 19 and CCL21, that are produced by stromal cells in the T cell zone of the spleen, lymph nodes, and Peyer's patches (1–3). This enables naive T cells to migrate to T cell areas of lymphoid organs in search of Ag presented by DC (4, 5). We have previously demonstrated that CCR7 is down-regulated in murine CD8 T cells activated in vivo by lymphocytic choriomeningitis virus (LCMV) infection, and observed that these effector T cells accumulated in the red pulp but failed to enter white pulp areas in the spleen (6). In humans, CCR7 has been described as a defining factor for two different types of memory T cells, termed central and effector memory T cells (7). Central memory cells express CCR7 and represent a nonpolarized Ag-experienced cell population that lacks immediate effector cell functions. In contrast, effector memory cells have down-regulated CCR7 and are capable of immediately producing cytokines after Ag recognition. This novel classification of memory T cells has attracted much attention and is widely cited in the current literature. Due to the lack of CCR7-specific mAb in mice, central and effector memory T cells defined by CCR7 expression have been studied almost exclusively in humans. In this study, we used a CCL19-Ig fusion protein to monitor CCR7 expression and compared immediate effector cell function of Ag-specific CCR7$^+$ and CCR7$^-$ murine T cells induced by a viral infection in vivo.

Materials and Methods

Mice

C57BL/6 (B6) mice were obtained from our breeding colony or from Harlan Winkelmann (Borchen, Germany). P14 TCR-tg (line 318) specific for LCMV gp33 plus H-2D^b (8), SMARTA TCR-tg mice specific for the LCMV gp61 plus I-A^b (9), and CCR7-deficient mice (5) on a mixed 129/B6 background have been described previously. Animals were kept under conventional conditions and were used for experiments at 8–16 wk of age.

Virus and peptides

The LCMV-WE used in this study was originally obtained from R. Zinkernagel (University Hospital, Zurich, Switzerland) and was propagated on L929 fibroblast cells. Mice were infected i.v. with 200 pfu of LCMV-WE. The LCMV glycoprotein peptides aa 33–41 (gp33 peptide, KAVYN FATM), aa 61–80 (gp61 peptide, GLNGPDIYKGVYQFKSVEFD), and the control adenovirus peptide E1A$_{234-243}$ (SGPSNTPPEI) were purchased from Neosystem (Strasbourg, France).

Flow cytometry

Lymphocytes were resuspended in PBS containing 2% FCS and 0.1% NaN_3 at a concentration of 10^6–10^7 cells/ml, followed by incubation at 4°C for 20 min with 100 μl of mAb at the working dilution. For PBL staining, 10 U/ml heparin was added to the staining buffer. To detect CCR7 cell surface expression, cells were incubated with COS cell supernatant containing ~1 μg/ml CCL19-Ig at 4°C for 60 min followed by biotinylated polyclonal anti-human Fcγ Abs (Dianova, Hamburg, Germany) and allophycocyanin-streptavidin (BD PharMingen, San Diego, CA). The chimeric CCL19-Ig fusion protein was generated as described (10) with minor modifications (S. Krautwald, E. Ziegler, R. Förster, L. Ohl, L. Renders, and U. Kunzendorf, manuscript in preparation). For intracellular cytokine staining, responder spleen cells (2×10^6) were cultured for 5 h with B6 stimulator spleen cells (2×10^6) loaded with gp33 or gp61 peptide (1 h, 37°C, 10^{-6} M) in 24-well plates. Afterward, cells were surface-stained with FITC-conjugated anti-Thy1.1 (clone OX-7) and CCL19-Ig, fixed, permeabilized, and stained intracellularly with PE-conjugated anti-IFN-γ (clone XMG1.2) or anti-TNF (clone MP6-xT22). Abs were purchased from BD PharMingen. Before analysis of PBL, red blood cells were lysed using FACS-Lysing Solution (BD PharMingen). Cells were analyzed on a FACSort flow

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Received for publication February 1, 2002. Accepted for publication May 16, 2002.

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[1] This work was supported by Deutsche Forschungsgemeinschaft Grant Pi-295/4-1.

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[3] Abbreviations used in this paper: CCL, CC chemokine ligand; LCMV, lymphocytic choriomeningitis virus.

0022-1767/02/$02.00

cytometer (BD Biosciences, Mountain View, CA) using CellQuest (BD Biosciences) software.

Isolation of liver T cells

Livers were perfused with PBS via the portal vein, excised, cut into small pieces, and digested in PBS containing 0.1% collagenase (Sigma-Aldrich, Munich, Germany), 0.01% hyaluronidase (Sigma-Aldrich), and 0.002% DNase I (Sigma-Aldrich) for 30 min at 37°C before being forced through a 100-μm cell strainer. Clumps and undigested material were allowed to settle and the resulting suspension was underlayed with Ficoll-Paque Plus (Amersham Pharmacia, Uppsala, Sweden) and cells from the interface were washed twice before further analysis.

Adoptive cell transfer and CTL assay

Spleen cells containing 10^5 naive TCR-tg cells from SMARTA- or P14-tg mice were adoptively transferred (i.v.) into B6 mice followed by LCMV infection. P14 TCR-tg cells, stained with CCL19-Ig and anti-Thy1.1 mAb, were sorted on a high-speed cell sorter (MoFlo; Cytomation, Fort Collins, CO) into Thy 1.1^+ CCL19-Ig^- and Thy 1.1^+ CCL19-Ig^+ populations (cell purity >80%). The cytolytic activity was determined in a 5-h ^{51}Cr release assay using EL-4 target cells as described (8).

Results

Kinetics of CCR7 surface expression on in vivo-activated CD8 T cells

To determine CCR7 cell surface expression levels by flow cytometry, a chimeric CCL19-Ig fusion protein was used. CD8 T cells from wild-type mice that had not undergone deliberate immunization (naive) were brightly stained by CCL19-Ig, whereas a large portion of activated CD8 T cells from LCMV-infected mice could no longer be stained with this reagent (Fig. 1*a*, *top row*). To further validate the use of CCL19-Ig to monitor CCR7 expression, CD8 T cells from CCR7-deficient mice were examined. As depicted in Fig. 1*a*, *bottom row*, CD8 T cells from both naive and LCMV-infected CCR7-deficient mice could not be stained with CCL19-Ig. These results indicate that CCR7 expression on T cells can be monitored by CCL19-Ig staining. In addition, other potential CCL19-binding receptors (e.g., CCR11; Ref. 11) do not appear to be expressed on these cells.

To generate effector/memory CD8 T cells with a defined Ag specificity in vivo, an adoptive transfer system with T cells from TCR-tg mice was used. Thy 1.1^+ CD8 T cells (10^5) from P14 TCR-tg mice specific for LCMV glycoprotein epitope gp33–41 in the context of H-$2D^b$ were adoptively transferred into B6 mice (Thy1.2) followed by LCMV infection. Donor cells were traced in the recipient mice using Thy1.1 as a marker for P14 TCR-tg cells. As shown in Fig. 1*b*, the LCMV infection induced vigorous expansion of the Thy 1.1^+ P14 TCR-tg cells peaking on day 8 after infection. Thereafter, the number of P14 TCR-tg cells declined to reach stationary levels 4–5 wk after infection.

Similar to normal mice, P14 TCR-tg cells from uninfected mice were uniformly stained with CCL19-Ig, indicating that CCR7 was expressed on most naive T cells at a high level (Fig. 1*d*, *left*). In contrast, CCL19-Ig staining separated P14 TCR-tg cells isolated 8 days after infection into $CCR7^+$ and $CCR7^-$ cell populations (Fig. 1*d*, *middle*). Interestingly, 5 wk after infection most P14 TCR-tg cells stained brightly with CCL19-Ig (Fig. 1*d*, *right*). This could indicate that memory T cells which differentiate from $CCR7^-$ effector cells up-regulate CCR7 or, alternatively, that $CCR7^-$ effector T cells did not survive the contraction phase of the anti-viral immune response.

CCR7 expression vs cytokine production of Ag-experienced CD8 T cells

The dot plots in Fig. 2*a* display CCL19-Ig vs intracellular IFN-γ staining, gated on Thy 1.1^+ P14 TCR-tg cells isolated from spleen

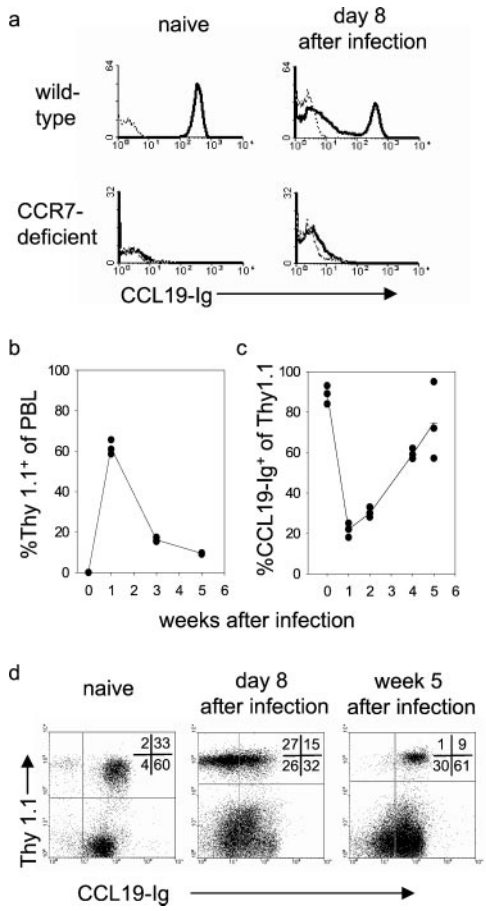

FIGURE 1. Down-regulation of CCR7 surface expression on CD8 T cells from LCMV-infected mice. *a*, CCL19-Ig staining (solid line) gated on CD8 T cells from PBL of uninfected and LCMV-infected wild-type and CCR7-deficient mice. Dashed lines represent negative staining controls. *b–d*, Kinetics and CCR7 expression of P14 TCR-tg effector/memory cells in vivo. Thy 1.1^+ P14 TCR-tg cells were transferred into B6 mice followed by LCMV infection. At the indicated time points after infection, the percentage of donor Thy 1.1^+ TCR-tg cells of total PBL (*b*) and the percentage of CCL19-Ig^+ of splenic Thy 1.1^+ cells (*c*) was determined. *d*, CCL19-Ig staining of Thy 1.1^+ P14 TCR-tg cells from uninfected mice (naive) and from recipients of TCR-tg cells 8 day and 5 wk after LCMV infection.

of mice that had not undergone deliberate immunization (naive) and from LCMV-infected recipient mice of P14 TCR-tg cells. Short-term (5 h) gp33 peptide stimulation induced 80–90% of P14 TCR-tg cells from LCMV-infected recipients, but not from uninfected mice, to produce high levels of IFN-γ. Without stimulation, P14 TCR-tg cells did not produce IFN-γ. Most importantly, both $CCR7^-$ and $CCR7^+$ subsets of P14 TCR-tg cells isolated 8 day or 4 wk after infection produced identical amounts of IFN-γ. A similar effect was seen when TNF production was determined in $CCR7^-$ and $CCR7^+$ subsets of P14 TCR-tg cells (Fig. 2*b*).

Recent reports have demonstrated that Ag-specific memory T cells also reside in nonlymphoid organs long after priming (12, 13). Therefore, we also examined CCR7 expression and IFN-γ secretion of P14 TCR-tg cells isolated from perfused livers of recipient mice 4 wk after LCMV infection (Fig. 2*c*). The analysis revealed two important points. First, CCR7 was expressed on about half of these cells and, second, both $CCR7^-$ and $CCR7^+$ subsets produced comparable amounts of IFN-γ.

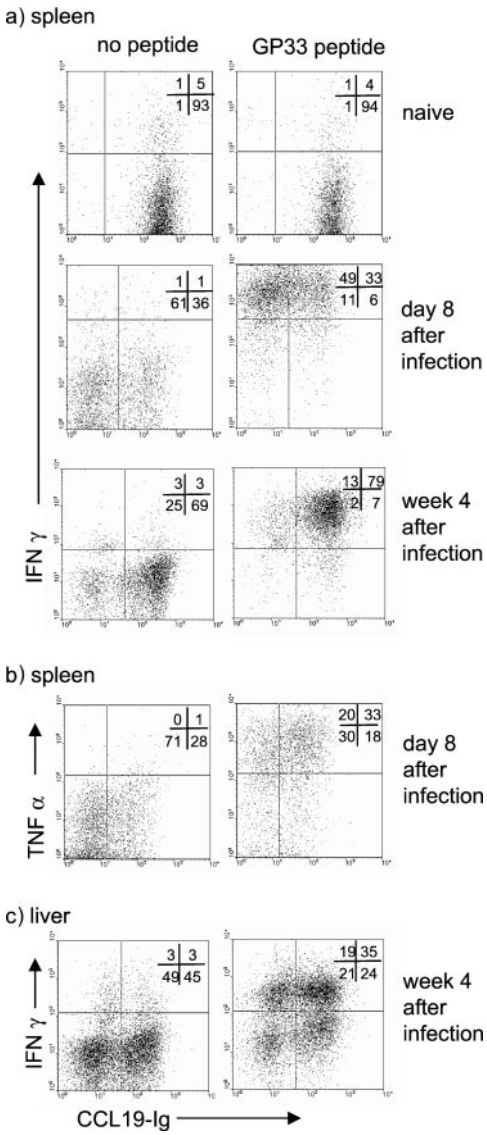

FIGURE 2. CCR7 expression vs cytokine production of in vivo activated P14 TCR-tg CD8 T cells. Thy1.1$^+$ P14 TCR-tg cells were transferred into B6 mice followed by LCMV infection. *a*, CCL19-Ig vs intracellular IFN-γ staining gated on Thy1.1$^+$ P14 TCR-tg cells from spleen of mice that had not undergone deliberate immunization (naive) and from recipient mice of TCR-tg cells 8 day and 4 wk after LCMV infection. *b*, CCL19-Ig vs intracellular TNF staining gated on Thy1.1$^+$ P14 TCR-tg cells from spleen of recipient mice 8 day after LCMV infection. *c*, CCL19-Ig vs intracellular IFN-γ staining gated on Thy1.1$^+$ P14 TCR-tg cells isolated from perfused livers of recipient mice 4 wk after LCMV infection.

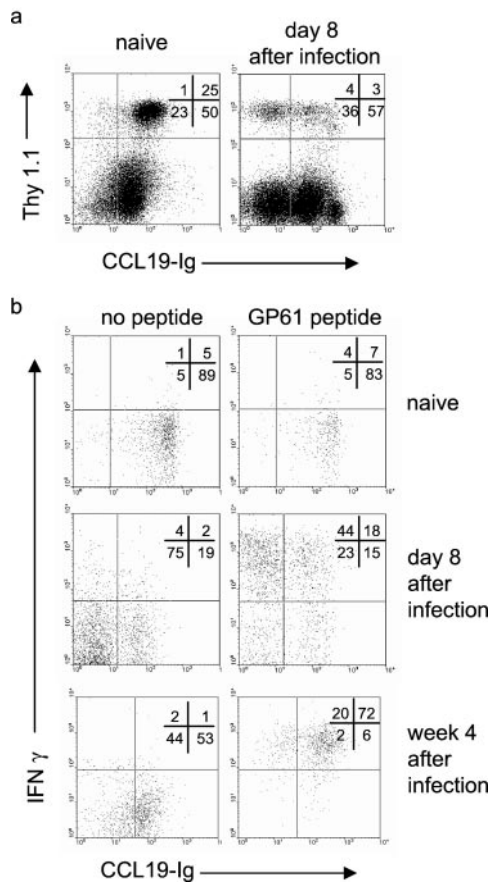

FIGURE 3. CCR7 expression vs IFN-γ production of in vivo-activated SMARTA TCR-tg CD4 T cells. Thy1.1$^+$ SMARTA TCR-tg cells were transferred into B6 mice followed by LCMV infection. *a*, CCL19-Ig staining of Thy1.1$^+$ SMARTA TCR-tg cells from uninfected mice (naive) and from recipients of TCR-tg cells 8 day after LCMV infection. *b*, CCL19-Ig vs intracellular IFN-γ staining gated on Thy1.1$^+$ SMARTA TCR-tg cells from mice that had not undergone deliberate immunization (naive) and from recipient mice of SMARTA TCR-tg cells 8 days and 4 wk after LCMV infection.

CCR7 expression vs cytokine production of Ag-experienced CD4 T cells

To trace LCMV-specific CD4 T cells in vivo, Thy1.1$^+$ CD4 T cells (10^5) from SMARTA TCR-tg mice specific for LCMV glycoprotein epitope gp61–80 in the context of I-A^b were adoptively transferred into B6 mice followed by LCMV infection. As in the CD8 transfer system, donor cells were traced in the recipient mice via the Thy1.1 marker. Thy1.1$^+$ SMARTA TCR-tg cells expanded in the infected recipient mice and represented up to 10% of total spleen cells in the acute phase of the infection. CCL19-Ig staining also separated SMARTA TCR-tg CD4 T cells into CCR7$^+$ and CCR7$^-$ cell populations (Fig. 3*a*). Intracellular cytokine staining further revealed that a substantial portion of SMARTA TCR-tg cells from acutely (day 8) LCMV-infected, but not from naive, mice produced IFN-γ after short-term stimulation with gp61 peptide. Importantly, IFN-γ production did not correlate with CCR7 expression, since IFN-γ secreting cells were present in both CCR7$^+$ and CCR7$^-$ subsets at similar relative percentages. (Fig. 3*b*, *middle panel*). The same conclusion was reached when SMARTA TCR-tg cells from mice 4 wk after LCMV infection were analyzed (Fig. 3*b*, *bottom panel*). At this later time point, CCR7 was expressed in a higher percentage (60–70%) of SMARTA TCR-tg cells when compared with day 8 after infection.

CCR7 expression vs immediate cytolytic activity

Besides cytokine production, cell-mediated lysis belongs to the key effector cell functions of activated CD8 T cells. Perforin protein expression in human CD8 T cells has been shown to be restricted to the CCR7$^-$ subset (7). The transfer system with CD8 T cells from P14 TCR-tg mice allowed us to directly correlate CCR7 expression with Ag-specific cytolytic activity. CCR7$^+$ and CCR7$^-$ P14 TCR-tg cells from LCMV-infected recipient mice were purified by cell sorting and were examined in ^{51}Cr release assays using LCMV gp33 peptide-coated target cells. As expected, P14 TCR-tg cells isolated 2 wk after infection exhibited a higher cytolytic activity on a cell-per-cell basis when compared with

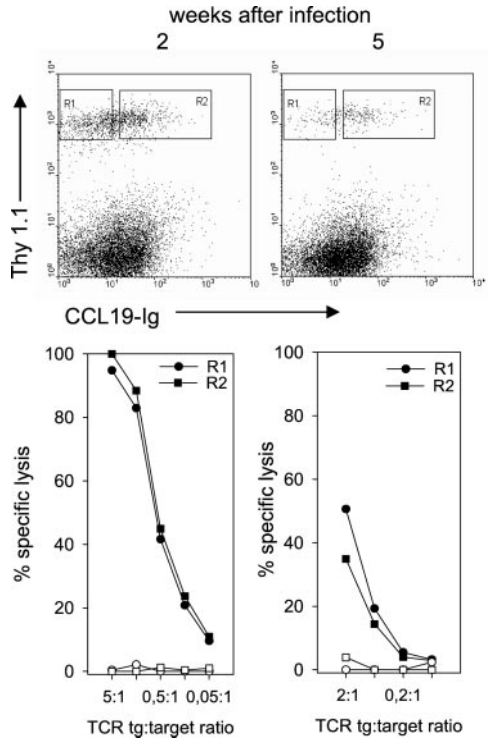

FIGURE 4. CCR7 expression vs cytolytic activity of in vivo-activated P14 TCR-tg CD8 T cells. Thy1.1$^+$ P14 TCR-tg cells were transferred into B6 mice followed by LCMV infection. Two and 5 wk after infection, Thy1.1$^+$ P14 TCR-tg cells were separated by high-speed cell sorting into CCL19-Ig$^-$ (R1, circles) and CCL19-Ig$^+$ (R2, quadrants) cell populations as depicted. The cytolytic activity was tested in a 5-h ^{51}Cr release assay using EL-4 target cells coated with LCMV gp33 (filled symbols) or control adenovirus peptide (open symbols). Percent specific lysis at the indicated TCR-tg to target cell ratio is shown.

TCR-tg cells from recipient mice 5 wk postinfection. At both time points, however, CCR7$^+$ and CCR7$^-$ P14 TCR-tg cells did not differ in LCMV gp33-specific cytolytic activity on a cell-per-cell basis (Fig. 4). Cross-linking of Thy1.1/CCR7 on P14 TCR-tg cells during the cell sorting procedure did not induce increased CTL activity, because anti-Thy1.1/CCL19-Ig-treated P14 TCR-tg memory cells produced the same degree of specific lysis of gp33 peptide-coated EL-4 target cells as untreated P14 memory T cells (data not shown). Thus, CCR7 surface expression on in vivo activated CD8 T cells did not correlate with their ability to lyse target cells.

Discussion

In summary, our data demonstrate that CCR7$^-$ and CCR7$^+$ memory T cells generated in vivo by a viral infection differed neither in cytokine production nor in cytolytic activity. In addition, a substantial number (~50%) of memory T cells isolated from livers of LCMV-infected recipient mice expressed CCR7. These findings do not fit into the current concept of central and effector memory cells originally proposed by Lanzavecchia and colleagues (7). These authors found that memory T cells expressing CCR7, termed central memory cells, were defective in immediate effector function. How can the conflicting data be explained? The concept of central and effector memory T cells was based on the analysis of human T cells whereas our data were derived from murine T cells. Therefore, it is possible that differences in memory T cell development between humans and mice exist. However, immediate IFN-γ production has also been observed in CCR7$^+$ memory-phenotype CD4 T cells from humans after PMA/ionomycin stimulation (14). Among Ag-specific human CD8 T cells defined with MHC class I tetramers, IFN-γ-producing cells have been found both in CCR7$^-$ and CCR7$^+$ subsets of HIV-infected individuals (15). Thus, these data, together with the present study, indicate that CCR7$^+$ memory T cells have the capacity to perform immediate effector cell functions both in humans and mice.

Acknowledgments

We thank Drs. S. Batsford and S. Ehl for comments on the manuscript, Tobias Junt (University Hospital, Zurich, Switzerland) for providing PBL from CCR7 knockout mice, Sabine Mac Nelly for help in liver perfusion and Theresa Treuer, Rainer Bronner, and Thomas Imhof for animal husbandry.

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