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Revealing the In Vivo Behavior of CD4⁺ T Cells Specific for an Antigen Expressed in *Escherichia coli*

Zong-ming Chen and Marc K. Jenkins

The clonal expansion and anatomic location of microbe-specific CD4⁺ Th cells was studied by tracking the fate of adoptively transferred DO11.10 TCR transgenic T cells specific for OVA peptide 323–339-I-A^d^ in BALB/c mice infected s.c. with *Escherichia coli* expressing a MalE-OVA fusion protein. After infection, the DO11.10 T cells accumulated in the T cell-rich paracortical regions of the draining lymph nodes, proliferated there for several days, and then moved into the B cell-rich follicles before they slowly disappeared from the lymph nodes. These changes occurred despite the fact that viable organisms were never found in the lymph nodes. The DO11.10 T cells also accumulated in the s.c. infection site, but about 1 day later than in the draining lymph nodes. Injection of purified MalE-OVA fusion protein alone induced a transient accumulation of DO11.10 T cells in the paracortical regions, but these T cells never entered follicles and the mice did not produce anti-OVA antibodies. The DO11.10 T cells that survived in animals injected with MalE-OVA alone were hyporesponsive to in vitro Ag restimulation and did not produce IL-2 and IFN-γ, whereas DO11.10 T cells from mice infected with MalE-OVA-expressing bacteria produced both lymphokines. These results suggest that Ag-specific T cells are first activated in secondary lymphoid organs following primary bacterial infection and then migrate to the infection site. Furthermore, productive activation of the T cells during the primary response is dependent on bacterial components other than the Ag itself. *The Journal of Immunology*, 1998, 160: 3462–3470.

**Materials and Methods**

**Bacterial strains and plasmids**

All strains used in this study were produced by transformation of *E. coli* strain DH5α (GIBCO/BRL, Grand Island, NY) with plasmids containing an ampicillin-resistance gene. The pMAL-p2 plasmid was purchased from New England Biolabs (Beverly, MA). The pAC-Neo-OVA plasmid (7) was kindly provided by Dr. Michael Bevan (University of Washington, Seattle, WA). The construction of plasmids used for this study is described below.

**Construction of a MalE-OVA fusion gene**

The pAC-Neo-OVA plasmid was used as a template for PCR amplification of a segment of the chicken OVA gene. The forward primer for the PCR was 5’-GAATCTAGAGCAGAGGCTGAGG-3’ that encodes an XhoI restriction site and amino acids 319 to 324 of chicken OVA, and the reverse primer was 5’-GGCGGTATCTTAAAGGGAAAACAT-3’ (Integrated DNA Technologies, Coralville, IA) that is complementary to the sequence that encodes residues 403 to 406 of chicken OVA plus 13 additional nucleotides. The PCR product DNA was then purified and ligated into the cloning site of the pCR II vector (a TA cloning vector) (Invitrogen, Carlsbad, CA) by taking advantage of the A-overhangs present at the ends of the PCR product and the T-overhangs present in the vector. Digestion of this plasmid with XbaI and HindIII produced a DNA fragment that was purified and ligated into the multiple clone site of the pMAL-p2 expression vector, which had been digested with the same pair of restriction enzymes. The resulting plasmid (pMAL-OVA) encodes a fusion protein with MalE at its N terminus and the last 87 amino acids of OVA at the C terminus.
was then quantitated using a Bio-Rad protein assay (Bio-Rad Laboratories, Beverly, MA). The purified MalE-OVA protein determined to be less than 50 ng/ml by the Chrome assay (Associates of Cape Cod). The final endotoxin concentration was determined by eluate from the affinity column with END-X B15 beads (Associates of Cape Cod, Woods Hole, MA). Residual endotoxin was removed by incubating the MalE-OVA fusion protein was enriched on an amylose affinity column overnight with IPTG as described above. Bacteria were induced with IPTG as described above. One million cells per ml of PBS s.c. into three sites on the back of each recipient mouse. In some cases, heat-inactivated bacteria (prepared by incubating bacteria in an 80°C water bath for 20 min) were used. Purified MalE-OVA fusion protein was enriched on an amylose affinity column according to the protocol provided by the manufacturer (New England Biolabs, Beverly, MA). Residual endotoxin was removed by incubating the eluate from the affinity column with END-X B15 beads (Associates of Cape Cod, Woods Hole, MA). The final endotoxin concentration was determined to be less than 50 ng/ml by the Limulus amebocyte lysate pyrochrome assay (Associates of Cape Cod). The purified MalE-OVA protein was then quantitated using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

**Immunoblotting**

Bacteria were induced with IPTG as described above. One million cells were lysed in 2× sample buffer (6% Tris-Cl, pH 6.8, 20% glycerol, 4% SDS, 2% 2-ME), and the lysates were separated on a 12% SDS polyacrylamide gel. Proteins were then transferred to nylon membranes, which were incubated overnight with blocking buffer (PBS with 0.05% Tween 20 and 5% BSA) at 4°C. The membranes were washed with 0.05% Tween 20 in PBS and incubated with a 1:2500 dilution of anti-OVA rabbit serum (Sigma Chemicals) at room temperature for 2 h with continuous shaking. Horseradish peroxidase-conjugated goat anti-rabbit serum (Sigma Chemicals, used at 1:10,000 dilution) was used as secondary Ab. The blot was developed using ECL reagents (Amersham International, Little Chalfont, U.K.).

**Ag injections**

*E. coli* containing the pMAL-OVA or pMAL-p2 plasmids were induced in vitro with IPTG as described above. Bacterial cells (10^8) were injected in 0.15 ml of PBS s.c. into three sites on the back of each recipient mouse. In some cases, heat-inactivated bacteria (prepared by incubating bacteria in an 80°C water bath for 20 min) were used. Purified MalE-OVA fusion protein (1 μg) was added to the culture to a final concentration of 0.3 mM. The culture was incubated for an additional 2.5 h before the bacteria were harvested by centrifugation at 4000 × g for 20 min. Periplasmic proteins were prepared by the osmotic shock method, and the MalE-OVA fusion protein was enriched on an amylose affinity column according to the protocol provided by the manufacturer (New England Biolabs, Beverly, MA). Residual endotoxin was removed by incubating the eluate from the affinity column with END-X B15 beads (Associates of Cape Cod, Woods Hole, MA). The final endotoxin concentration was determined to be less than 50 ng/ml by the Limulus amebocyte lysate pyrochrome assay (Associates of Cape Cod). The purified MalE-OVA protein was then quantitated using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

**Recombinant**

*E. coli* DH5α containing pMAL-OVA or pMAL-p2 were grown in Luria-Bertani broth supplemented with 100 μg/ml ampicillin and 2 mg/ml glucose overnight at 37°C. The overnight culture was diluted 1:100 into fresh Luria-Bertani broth supplemented with ampicillin and glucose and grown at 37°C to 2 × 10^8 cells/ml. Isopropyl-β-D-thiogalactoside (IPTG; Sigma, St. Louis, MO) was added to the culture to a final concentration of 0.3 mM. The culture was incubated for an additional 2.5 h before the bacteria were harvested by centrifugation at 4000 × g for 20 min. Periplasmic proteins were prepared by the osmotic shock method, and the MalE-OVA fusion protein was enriched on an amylose affinity column according to the protocol provided by the manufacturer (New England Biolabs, Beverly, MA). Residual endotoxin was removed by incubating the eluate from the affinity column with END-X B15 beads (Associates of Cape Cod, Woods Hole, MA). The final endotoxin concentration was determined to be less than 50 ng/ml by the Limulus amebocyte lysate pyrochrome assay (Associates of Cape Cod). The purified MalE-OVA protein was then quantitated using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

**Mice and adoptive transfer**

BALB/c mice were purchased from the National Cancer Institute (Frederick, MD). The DO11.10 TCR transgenic mice (9) were bred in a specific pathogen-free facility according to National Institutes of Health guidelines and screened for transgene expression as previously described (6). These mice have been extensively backcrossed (>15 generations) onto the BALB/c background and are therefore histocompatible with normal BALB/c mice. Adoptive transfer was performed as previously described (6). Briefly, pooled splenocytes and lymph node cells from DO11.10 donors were depleted of CD8+ T cells by Ab plus complement treatment, and 2.5 × 10^9 T cells present in the surviving population was determined in an aliquot of the cells by two-color flow cytometry as described below. Cells from an unstained aliquot were then injected into unirradiated BALB/c mice such that each mouse received 2.5 × 10^9 CD4+ and 2 × 10^6 CD4- T cells.
Values for the total number of CD4+ total number of cells in the relevant lymph nodes. The total number of CD4+ and KJ1-26+ is indicated on the plot. The stained lymph node cells were from normal BALB/c mice (A), BALB/c mice injected with 2.5 × 10^6 DO11.10 T cells (B), BALB/c recipients of DO11.10 T cells 3 days after s.c. infection with 10^8 MalE-OVA-expressing E. coli (C), and BALB/c recipients of DO11.10 T cells 3 days after s.c. infection with 10^8 control E. coli (D).

10^6 lymph node cells were incubated on ice with phycoerythrin (PE)-labeled anti-CD4 mAb (PharMingen, San Diego, CA) and biotinylated KJ1-26 mAb, which recognizes the DO11.10 TCR and no other (11). Cells were then washed and incubated with FITC-labeled streptavidin (SA; Caltag, South San Francisco, CA) to detect the KJ1-26 mAb. Two thousand events were then collected for each sample on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and analyzed using Lysis II software (Becton Dickinson). DO11.10 cells were identified as CD4+, KJ1-26+ cells. The total number of DO11.10 T cells present at any given time was calculated by multiplying the total number of viable lymph node cells (obtained by counting viable cells) by the percentage of CD4+, KJ1-26+ cells obtained by flow cytometry.

Cell surface markers and BrdU- or propidium iodide-labeled DNA were detected by three-color flow cytometry. Surface markers were detected in cells stained with anti-CD4-PE mAb, biotinylated KJ1-26 mAb, SA-CyChrome, and either FITC-labeled anti-LFA-1, i-selectin, or isotype-matched control mAb (all purchased from PharMingen). BrdU-labeled DNA was detected in lymph node cells stained with anti-CD4-PE mAb, biotinylated KJ1-26 mAb and SA-CyChrome, incubated with 1% paraformaldehyde and 0.01% Tween 20, and stained with FITC-labeled anti-BrdU mAb as described by Carayon and Bord (12). Propidium iodide-labeled DNA was detected in cells stained with anti-CD4-PE mAb, biotinylated KJ1-26 mAb and SA-CyChrome, fixed with 70% ethanol, and stained with propidium iodide. In each case, the CD4+, KJ1-26+ cells were identified on a FACScan flow cytometer, and the fluorescence intensities of 1000 of these cells in the third color were measured.

Immunohistochemistry

Brachial lymph nodes were flash frozen in OCT embedding media (Miles, Elkhart, IN). Thin sections (6 µm) were cut on a refrigerated microtome and fixed to glass slides with acetone. Peroxidase-labeled KJ1-26 or anti-B220 mAb were used to detect DO11.10 T cells or B cells, respectively, as previously described (13).

In vitro culture

Lymph node cells (2 × 10^6) were cultured with 5 × 10^5 irradiated (3000 rads) BALB/c splenocytes and 5 µM OVA peptide 323–339 in 0.2 ml of complete Eagle’s Hanks’ amino acids medium (Biofluids, Rockville, MD) supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, 20 mg/ml gentamicin sulfate, and 5 × 10^-5 M 2-ME for 60 h, and then the last 12 h in the presence of 1 µCi of [3H]thymidine. Alternatively, 2 × 10^6 cells from a short-term T cell line derived from DO11.10 mice were cultured for 72 h with heat-killed bacteria and 10^5 irradiated BALB/c peritoneal exudate cells as APC, the last 8 h in the presence of [3H]thymidine. The cultures were harvested at the end of the culture period and [3H]thymidine incorporation in DNA was measured by liquid scintillation counting.

Detection of IL-2 and IFN-γ by ELISA

IL-2 and IFN-γ in culture supernatants were measured by capture ELISA using noncompeting mAb pairs according to the protocol provided by the

**FIGURE 2.** Detection of transferred DO11.10 T cells in lymph node populations. Pooled brachial, axillary, and inguinal lymph node suspensions were stained with anti-CD4-PE and biotinylated KJ1-26, followed by SA-FITC, and analyzed on a flow cytometer. The percentage of lymphocytes that were CD4+ and KJ1-26+ is indicated on the plot. The stained lymph node cells were from normal BALB/c mice (A), BALB/c mice injected with 2.5 × 10^6 DO11.10 T cells (B), BALB/c recipients of DO11.10 T cells 3 days after s.c. infection with 10^8 MalE-OVA-expressing E. coli (C), and BALB/c recipients of DO11.10 T cells 3 days after s.c. infection with 10^8 control E. coli (D).

**FIGURE 3.** Kinetics of accumulation of DO11.10 T cells in lymph nodes. Brachial, axillary, and inguinal lymph node cells were pooled from 3 to 6 animals/group/time point on the indicated days following s.c. injection of nothing (○), 100 µg of purified MalE-OVA fusion protein only (△), 100 µg of purified MalE-OVA fusion protein mixed with 10^7 non-OVA-expressing control E. coli (▲), 10^8 E. coli expressing the MalE-OVA fusion protein (●), or 10^8 control E. coli (□). The percentage of CD4+, KJ1-26+ T cells was determined by flow cytometry. The total number of CD4+, KJ1-26+ cells was determined by multiplying the percentage of CD4+, KJ1-26+ cells by the total number of cells in the relevant lymph nodes. Values for the total number of CD4+, KJ1-26+ cells × 10^-4 ± SEM are shown.
To measure serum anti-OVA IgG levels, serial dilutions of serum samples were incubated for 2 h at 37°C in EIA/RIA plates (Costar, Cambridge, MA) coated with OVA (20 μg/ml OVA for 3 h and blocked with 1% BSA for 1 h at 37°C). The plates were washed with 0.5% Tween 20 in PBS, and the bound Abs were detected with a 1:5000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (Pierce, Rockford, IL). O-phenylenediamine (Sigma) was used as the chromagen. The optical densities at 490 nm were determined, and the titer at half of the maximal optical density was calculated for each sample.

Results
Recombinant E. coli expressing a MalE-OVA fusion protein stimulate DO11.10 T cells in vitro

A recombinant E. coli strain was designed to express a fusion protein containing E. coli protein MalE at its N terminus and amino acids 319 to 406 of chicken OVA at its C terminus (Fig. 1A), under the control of the Ptac promoter. The expression of the fusion protein was confirmed by immunoblot analysis of lysates from IPTG-induced bacteria probed with a polyclonal antiserum containing anti-chicken OVA Abs (Fig. 1B). Based on densitometric comparison between immunoblots performed with bacterial lysates and purified OVA, the amount of fusion protein in the IPTG-induced recombinant bacteria was estimated to be about 1 μg OVA per 10^6 bacterial cells. To test whether a peptide-I-A<sup>d</sup> complex that could be recognized by DO11.10 T cells was produced from the MalE-OVA fusion protein by Ag processing, peritoneal macrophages were exposed to IPTG-induced recombinant bacteria and then tested in vitro for stimulation of DO11.10 T cells. As shown in Figure 1C, DO11.10 T cells proliferated in vitro in response to macrophages pulsed with bacteria expressing MalE-OVA.
but not MalE alone. Furthermore, the proliferation of DO11.10 T cells in response to MalE-OVA-expressing bacteria was blocked by addition of anti-I-Ad, but not anti-I-E\textsubscript{d} mAb (data not shown). Together, the results demonstrate that a peptide-I-A\textsubscript{d} complex capable of stimulating DO11.10 T cells was generated by APC exposed to recombinant \textit{E. coli} expressing the MalE-OVA fusion protein.

Characterization of the primary DO11.10 T cell response induced in vivo by \textit{E. coli} expressing the MalE-OVA fusion protein

The capacity of recombinant \textit{E. coli} expressing the MalE-OVA fusion protein to stimulate OVA-specific T cells in vivo was assessed with the adoptive transfer method we used previously to characterize the primary T cell response induced by soluble OVA (6). A small number of CD4\textsuperscript{+}, OVA peptide 323–339-I-A\textsubscript{d}-specific T cells from the DO11.10 TCR transgenic mouse line were transferred into normal BALB/c recipients. Flow cytometry was then used to identify the transferred T cells following staining of recipient lymph node cells with the KJ1-26 anti-clonotypic mAb, which uniquely binds to the DO11.10 TCR. CD4\textsuperscript{+}, KJ1-26\textsuperscript{+} cells were detected in the lymphoid tissues of BALB/c mice previously injected with DO11.10 T cells (Fig. 2B) but not in normal BALB/c mice that did not receive DO11.10 T cells (Fig. 2A). A s.c. injection of \(10^8\) live \textit{E. coli} expressing the MalE-OVA fusion protein caused the DO11.10 T cells to become more numerous in the draining lymph nodes (Fig. 2C) compared with injection of nothing (Fig. 2B) or control \textit{E. coli} expressing MalE alone (Fig. 2D). Analysis of the kinetics of this response revealed that as early as day 3 postinfection, the number of DO11.10 T cells in the draining lymph nodes was significantly higher in mice infected with MalE-OVA-expressing bacteria than in mice infected with control bacteria (Fig. 3). The number of DO11.10 T cells in mice infected with MalE-OVA-expressing bacteria continued to increase to a peak on day 5 and then declined slightly by day 7, and dramatically by day 17.

The majority of the DO11.10 T cells (identified as shown in Fig. 4) in mice infected 2 days before with MalE-OVA-expressing \textit{E. coli} were blasts (Fig. 4B). In addition, many of the DO11.10 T cells recovered from the lymph nodes of mice infected with MalE-OVA-expressing bacteria possessed the surface phenotype indicative of prior activation (14): L-selectin\textsuperscript{low}, LFA-1\textsuperscript{high} (Fig. 4, C and D); whereas DO11.10 T cells recovered from mice infected with control bacteria had the surface phenotype of naive T cells: L-selectin\textsuperscript{high}, LFA-1\textsuperscript{low}. On day 3 postinfection, significantly more DO11.10 cells were in the S, G\textsubscript{2}, or M phases of the cell cycle in the lymph nodes of mice infected with MalE-OVA-expressing bacteria than in comparable nodes of mice infected with control bacteria (Fig. 3). The percentages of CD4\textsuperscript{+}, KJ1-26\textsuperscript{+} cells in the S, G\textsubscript{2}, or M phases of the cell cycle defined by the M1 gate are indicated. B, Anti-BrdU (solid line) or an isotype-matched control mAb (dashed line) staining of CD4\textsuperscript{+}, KJ1-26\textsuperscript{+} cells present in lymph nodes 5 days after infection of mice continuously exposed to BrdU in the drinking water. The percentages of BrdU-positive cells present in the M1 gate are shown.
and although a few CD4+ KJ1-26+ DO11.10 T cells were present in the air sacs of mice infected with MalE-OVA-expressing (Fig. 7, D and G), but not control, bacteria (Fig. 7, E and G). DO11.10 T cells accumulated in the draining lymph nodes about a day earlier than they appeared at the infection site, achieving a near maximal level by day 3 postinfection (Fig. 7G). This clonal expansion of the DO11.10 T cells in the lymph nodes occurred despite the fact that viable bacteria were not detected in the draining lymph nodes at any time after infection (Fig. 6). Taken together, the data support the possibility that Ag-specific T cells initially proliferate in the secondary lymphoid tissues in response to debris carried from the infection site in the afferent lymph, increase expression of molecules involved in extravasation such as LFA-1, and then migrate to the nonlymphoid infection site.

Injection of purified MalE-OVA fusion protein in the absence of other bacterial components results in DO11.10 T cell hyporesponsiveness

Previous work with this system showed that injection of soluble OVA alone caused the DO11.10 T cells to proliferate transiently in the T cell areas of the draining lymph nodes (6). Most of the cells then disappeared, and although the surviving cells had the surface phenotype of memory cells, they were hyporesponsive to antigenic stimulation (6). In contrast, injection of OVA plus the adjuvants CFA or LPS caused the DO11.10 T cells to proliferate more extensively in the T cell areas and migrate into the B cell-rich follicles (6, 13). Although many of the DO11.10 T cells again disappeared, many did not, and the surviving cells responded rapidly to Ag stimulation by producing lymphokines including IFN-γ as expected for memory cells (13).

Because adjuvants were required for maximal generation of memory T cells when soluble OVA was used as the Ag, it was of interest to determine whether the in vivo stimulation of DO11.10 T cells by the MalE-OVA fusion protein was influenced by the natural adjuvanticity of the bacteria. This was investigated by studying the in vivo behavior of DO11.10 T cells following injection of affinity-purified, endotoxin-free MalE-OVA protein. As shown in Figure 3, s.c. injection of the purified fusion protein caused the DO11.10 cells to accumulate rapidly in the draining lymph nodes to a peak level on day 3. However, most of the cells then disappeared rapidly from the lymph nodes. In addition, the number of DO11.10 T cells present on day 3 in the lymph nodes of mice injected with MalE-OVA alone was much lower than the number present in mice infected with MalE-OVA-expressing bacteria. Furthermore, the DO11.10 T cells did not accumulate in the B cell-rich follicles in response to soluble MalE-OVA (Fig. 8A) to the same extent as they did in response to MalE-OVA-expressing bacteria (Fig. 8C). Quantitative image analysis revealed that the density of DO11.10 T cells in the follicles 3 days after injection of MalE-OVA alone (Fig. 8A) was 14-fold lower than the density of DO11.10 T cells in the follicles of mice injected with MalE-OVA-expressing bacteria (Fig. 8C). Despite the fact that many of the DO11.10 cells became blasts 3 days after injection of soluble MalE-OVA (Fig. 4B) and many converted to a “memory” cell phenotype (Fig. 4, C and D), these proliferated poorly (Fig. 9B) and did not produce IL-2 (Fig. 9C) or IFN-γ (Fig. 9D) in response to in vitro stimulation with the OVA peptide at later times. These results indicate that soluble MalE-OVA stimulates a transient and abortive response by the DO11.10 T cells that does not produce functional memory cells.

To assess whether the productive T cell response only occurred when MalE-OVA was physically associated with the bacterial

Ag-activated T cells migrate to the nonlymphoid site of bacterial infection

The ability to physically track Ag-specific T cells allowed us to determine whether the initial activation of naive T cells occurs in the secondary lymphoid tissues or the nonlymphoid site where the Ag enters the body. We addressed this point by using the connective tissue air sac model of bacterial infection (10). Air sacs were produced in the skin on the backs of mice that previously received DO11.10 T cells and bacteria present within the air sac and the draining lymph nodes was measured. Viable bacteria were present in the air sac immediately after injection (Fig. 6). The number of viable bacteria in the air sac declined slowly after this; however, many viable bacteria were still present 6 days postinfection (Fig. 6). No difference in the rate of clearance of the MalE-OVA-expressing or control bacteria was observed (Fig. 6). From days 1 to 3 postinfection, most of the cells recovered from the air sacs were neutrophils and macrophages as assessed by expression of the Gr-1 and CD11b markers (Fig. 7A). A small number of cells had the light scatter properties of lymphocytes at this time (R1 in Fig. 7B), and although a few CD4+ KJ1-26+ cells of recipient origin were present in this population, few if any DO11.10 T cells were detected (Fig. 7, C and G). However, by day 4 postinfection a clearly defined population of CD4+, KJ1-26+ DO11.10 T cells was present in the air sacs of mice infected with MalE-OVA-expressing (Fig. 7, D and G), but not control, bacteria (Fig. 7, E and G).
cells, adoptive transfer recipients were injected with a mixture of soluble MalE-OVA protein and bacteria that do not express MalE-OVA. The DO11.10 T cells behaved under these conditions exactly as they did when confronted with MalE-OVA-expressing bacteria: the cells accumulated to high levels in the draining lymph nodes (Fig. 3), entered the follicles (data not shown), and differentiated into IL-2 (Fig. 9C) and IFN-γ-producing (Fig. 9D) memory T cells. In addition, recipients injected with soluble MalE-OVA plus control bacteria produced the same amount of anti-OVA Abs as mice infected with MalE-OVA-expressing bacteria (Fig. 9A). These results demonstrate that the bacteria did not have to express the OVA themselves to facilitate induction of productive immunity.

Discussion
The experiments presented here were designed to extend our previous studies on the in vivo behavior of Ag-specific CD4+ T cells to a more physiologic situation where the Ag of interest was expressed by a microbe. T cell responses induced by microbes might...
be expected to differ from those induced by soluble Ags because microbes are a source of replicating Ag and thus would not necessarily disappear rapidly from the body. In addition, microbial products may modify the T cell response, for example, by altering Ag processing or presentation (15) or T cell activation (16). Given these possibilities, it was remarkable to observe that MalE-OVA-expressing bacteria stimulated the same kinetics of clonal expansion and contraction, follicular migration, and differentiation of the Ag-specific T cells stimulated by injection of soluble Ag in CFA or LPS (6, 13). Therefore, it appears that T cell responses to microbial Ags, at least those derived from noninvasive microbes, can be reliably modeled with soluble Ags plus adjuvant.

FIGURE 8. Immunohistochemical analysis of lymph nodes from recipients of DO11.10 transgenic T cells. BALB/c recipients of DO11.10 T cells were injected s.c. with purified MalE-OVA fusion protein (A and B), MalE-OVA-expressing E. coli (C and D), or control E. coli (E and F), as described in Figure 4. Adjacent frozen sections were stained with KJ1-26 (A, C, and E) or B220 (B, D, and F) to delineate the B cell-rich follicles. Primary Abs were detected with the peroxidase method (6). Positively stained cells appear as black dots. Video images of the stained sections were captured and analyzed using Adobe Photoshop software. An outline was drawn around the follicular regions on the images from the sections stained with anti-B220. This outline was then copied and pasted onto the corresponding area of the image from the adjacent sections stained with KJ1-26.

FIGURE 9. Ab and lymphokine production after primary exposure to Ag in vivo. BALB/c recipients of DO11.10 T cells were injected s.c. with MalE-OVA-expressing E. coli (Group 1), purified MalE-OVA fusion protein (Group 2), or a mixture of purified MalE-OVA fusion protein and control E. coli (Group 3), as described in Figure 4. Fourteen days later, serum levels of OVA-specific IgG were measured by ELISA (A). The percentage of CD4⁺, KJ1-26⁺ cells in the brachial, axillary, and inguinal lymph node cells from individual mice in each treatment group was determined by FACS as described in Figure 1. Some of the remaining cells were cultured with irradiated BALB/c splenocytes and the OVA peptide 323–339 (the indicated doses in B, and 5 mM in C and D). T cell proliferation was measured by [³H]thymidine incorporation after 60 h of culture (B). IL-2 (C) and IFN-γ (D) production were measured by ELISA from 48-h supernatants of Ag-stimulated cultures. The amount of proliferation or lymphokine was corrected for the number of KJ1-26⁺ cells added to each well.
The simplest explanation for the finding that MalE-OVA stimulated a productive response by Ag-specific T cells when introduced into the body as part of a bacterium but not as a soluble protein is that bacterial components act as immunologic adjuvants (17). We have shown that efficient clonal expansion and follicular migration of Ag-activated T cells only occurs when Ag is delivered with LPS, TNF-α, or IL-1 (13). Thus, it is likely that bacterial components such as LPS stimulate TNF-α and IL-1 production by macrophages, and that these cytokines produce an environment that is conducive to T cell activation (18). This could be accomplished by induction of adhesion and costimulatory molecules (19) or stabilization of peptide-MHC molecules (20) on APC such as dendritic cells, the most abundant class II MHC-bearing APC in the T cell areas of lymphoid tissue (21). An alternative possibility was that the recombinant bacteria provide a replicating source of OVA, which results in prolonged Ag-presentation compared with a single injection of soluble OVA. However, the finding that a mixture of nonreplicating soluble Ag and control bacteria induced full activation of the Ag-specific T cells is strong evidence against this possibility.

Our results suggest that the T cells specific for microbial Ags are initially activated in the secondary lymphoid tissues such as the draining lymph nodes. This supposition is based on the finding that activated Ag-specific T cells accumulated in the draining lymph nodes before accumulating in the s.c. injection site. Earlier work in this system showed that naive DO11.10 T cells are only present in the blood and T cell-rich areas of secondary lymphoid tissues of recipient mice; few if any cells are found in nonlymphoid tissues (22). This restriction argues that naive T cells would have to be activated in lymphoid tissues before migrating to nonlymphoid tissues. The finding that viable bacteria were never found in the lymph nodes suggests that some of the bacteria are killed at the injection site, perhaps by neutrophils, and that debris including MalE-OVA is carried to the lymph nodes via the afferent lymph for presentation by resident APC. Alternatively, immature dendritic cells at the s.c. site may internalize bacterial debris, process MalE-OVA, and migrate to the draining lymph node (23–25). Once activated in the secondary lymphoid tissues, the Ag-specific T cells that had lost LSelectin and increased expression of LFA-1, changes that would be predicted to limit re-entry into lymph nodes and facilitate entry into inflamed nonlymphoid tissues such as the injection site (26, 27).

The finding that MalE-OVA-expressing and control bacteria were cleared equally well, despite the fact that the mice contained an elevated population of OVA-specific DO11.10 T cells, is consistent with work from other studies that showed that cells other than T cells are the primary effectors of clearance of E. coli (28). However, it is possible that T cells contribute to clearance, but the number of T cells that are specific for E. coli peptide-MHC complexes is not limiting such that the additional DO11.10 T cells made no difference. Although no role for the transferred T cells in clearance of E. coli could be shown here, adoptive transfer of T cells from bacterial Ag-specific TCR transgenic donors should be a powerful tool for studying the in vivo behavior of Ag-specific T cells during infections caused by pathogens such as Salmonella, protection from which is known to be dependent on CD4+ T cells (29).

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References


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