Coordinate Involvement of Invasin and Yop Proteins in a *Yersinia pseudotuberculosis* -Specific Class I-Restricted Cytotoxic T Cell-Mediated Response

Géraldine Falgarone, Hervé S. Blanchard, François Virecoulon, Michel Simonet and Maxime Breban

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Yersinia pseudotuberculosis is a pathogenic enteric bacteria that evades host cellular immune response and resides extracellularly in vivo. Nevertheless, an important contribution of T cells to defense against Yersinia has been previously established. In this study we demonstrate that Lewis rats infected with virulent strains of Y. pseudotuberculosis, mount a Yersinia-specific, RT1-A-restricted, CD8⁺ T cell-mediated, cytotoxic response. Sensitization of lymphoblast target cells for cytolysis by Yersinia-specific CTLs required their incubation with live Yersinia and was independent of endocytosis. Although fully virulent Yersinia did not invade those cells, they attached to their surface. In contrast, invasin-deficient strain failed to bind to blast targets or to sensitize them for cytolysis. Furthermore, an intact virulence plasmid was an absolute requirement for Yersinia to sensitize blast targets for cytolysis. Using a series of Y. pseudotuberculosis mutants selectively deficient in virulence plasmid-encoded proteins, we found no evidence for a specific role played by YadA, YopH, YpkA, or YopJ in the sensitization process of blast targets. In contrast, mutations suppressing YopB, YopD, or YopE expression abolished the capacity of Yersinia to sensitize blast targets. These results are consistent with a model in which extracellular Yersinia bound to lymphoblast targets via invasin translocate inside eukaryotic cytosol YopE, which is presented in a class I-restricted fashion to CD8⁺ cytotoxic T cells. This system could represent a more general mechanism by which bacteria harboring a host cell contact-dependent or type III secretion apparatus trigger a class I-restricted CD8⁺ T cell response. The Journal of Immunology, 1999, 162: 2875–2883.
bacterium by class I molecules is that such Ag presumably need to penetrate the cell to gain access to class I presentation pathway (18). To date, class I presentation has been shown to work efficiently for a limited number of bacteria that survive intracellularly, such as *Listeria*, *Mycobacteria*, *Salmonella*, and *Chlamydia* (19, 20).

In the present work we provide evidence that infection of rat with *Y. pseudotuberculosis*, an extracellularly living bacteria, elicits a bacteria-specific, class I-restricted, cytotoxic CD8* T cell response. The roles played by invasin and virulence plasmid-encoded proteins in this response are further characterized, and its functional implications are discussed.

**Materials and Methods**

**Rats**

Inbred Lewis (LEW), and Dark Agouti females, 2–3 mo old, were purchased from Iffa Credo (L’Arbresle, France) and housed under conventional conditions. Study procedures were approved by the institutional animal care committee.

**Bacterial strains and growth conditions**

*Y. pseudotuberculosis* strains (Table I) used in this study were grown in Luria broth (LB) medium, sometimes supplemented with 20 mM sodium oxalate/20 mM MgCl2 (Ca2+-deficient LB medium) at 28 or 37°C. Strains of *Escherichia coli* 25922 obtained from American Type Culture Collection (Manassas, VA) and *Yersinia* II. The anti-*Yersina* invasin mAb 3A2 (murine IgG2a) (39) was a gift from R. R. Isberg (Tufts University, Boston, MA). Irrelevant murine mAbs were γc11 (anti-human CD82, IgG1; a gift from H. Conjeaud, Paris, France) and OKT3 (anti-human CD3, IgG2a; Cilag, Boulogne, France). Goat anti-mouse IgG-FITC was purchased from Eurobio (Les Ulis, France). Cytochalasin D (CCD) was obtained from Sigma (St. Quentin Fallavier, France).

**Infection of animals**

*Y. pseudotuberculosis* grown overnight at 28°C in Ca2+-deficient LB medium were centrifuged and resuspended in sterile PBS. For intragastric infection, rats were given 109 bacteria in 0.5 ml of PBS through a gastric tube for 3 consecutive days. For i.p. infection, rats received a single inoculum of 106 bacteria in 0.5 ml of PBS.

**Generation of anti-*Yersinia* CTLs**

Peripheral and mesenteric LN cells harvested from infected rats were resuspended in culture medium containing 10% Con A-stimulated rat spleen and LN cell supernatant and 50 mM α-methyl-D-mannoside and restimulated for 4–5 days in 96-well culture dishes (105 cells/well) with LN cells from naive rats (× 104 cells/well) that had been in vitro infected with *Y. pseudotuberculosis* as follows. Bacteria grown overnight at 28°C in LB medium followed by 4-h incubation at 37°C in Ca2+-deficient LB medium to induce expression of virulence plasmid proteins were washed and resuspended in tissue culture medium without antibiotics and incubated with stimulator cells at a ratio of 10:90 bacteria/cell for 2 h at 37°C. Thereafter, infected stimulator cells were treated for 1 h at 37°C with gentamicin (100 µg/ml), washed, and irradiated (3000 rad) before use.

**Anti-H-Y CTLs**

CTLs specific for the rat male H-Y Ag were generated as previously described (40). Briefly, LEW females were primed in vivo by s.c. and i.p. injection of 2 × 107 LEW male pooled LN and spleen cells. LN cells harvested from LEW female primed >3 wk previously were resuspended in culture medium containing 10% Con A supernatant and 50 mM α-methyl-D-mannoside and restimulated for 5 days in 96-well U-bottom culture dishes (105 cells/well) with irradiated (3000 rad) LN cells from LEW male (× 105 cells/well).

**Table I. *Y. pseudotuberculosis* strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant Characteristic</th>
<th>Source or Ref.</th>
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<tbody>
<tr>
<td>IP277</td>
<td>Wild type, pYV+</td>
<td>5</td>
</tr>
<tr>
<td>IP277c</td>
<td>IP277 derivative, pYV-cured</td>
<td>5</td>
</tr>
<tr>
<td>YP11</td>
<td>Wild type, pYV- (pIB1)</td>
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</tr>
<tr>
<td>YP11c</td>
<td>YP11 derivative, pYV-cured</td>
<td>21</td>
</tr>
<tr>
<td>YP212</td>
<td>YP11 derivative, inv mut</td>
<td>22</td>
</tr>
<tr>
<td>YP15</td>
<td>YP11 derivative, yadA mut</td>
<td>23</td>
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<tr>
<td>YPII(pB15)</td>
<td>YPII derivative, yopE329-210</td>
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<tr>
<td>YP11(pB52)</td>
<td>YP11 derivative, yopE mut</td>
<td>25</td>
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<tr>
<td>YP11(pB43)</td>
<td>YP11 derivative, spkA384-387, yopI mut</td>
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<td>YP11(pB44)</td>
<td>YP11 derivative, spkA307-308 mut</td>
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<tr>
<td>YP11(pB601)</td>
<td>YP11 derivative, yopB yopD mut</td>
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<tr>
<td>YP11(pB604)</td>
<td>YP11 derivative, yopB yopD mut</td>
<td>28</td>
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<tr>
<td>YP11(pB605)</td>
<td>YP11 derivative, yopD mut</td>
<td>28</td>
</tr>
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**Table II. Murine anti-rat Ag mAbs used**

<table>
<thead>
<tr>
<th>mAb</th>
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<th>Ag</th>
<th>Cell Population Marked</th>
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<tbody>
<tr>
<td>R73</td>
<td>IgG1</td>
<td>αβ TCR</td>
<td>All αβ T cells</td>
<td>29</td>
</tr>
<tr>
<td>OX34</td>
<td>IgG2a</td>
<td>CD2</td>
<td>All rat T cells</td>
<td>30</td>
</tr>
<tr>
<td>OX8</td>
<td>IgG1</td>
<td>CD8 α-chain</td>
<td>T cell, NK cells</td>
<td>31</td>
</tr>
<tr>
<td>OX35</td>
<td>IgG2a</td>
<td>CD4</td>
<td>T cells, macrophages</td>
<td>32</td>
</tr>
<tr>
<td>OX33</td>
<td>IgG1</td>
<td>CD45 epitope</td>
<td>All B cells</td>
<td>33</td>
</tr>
<tr>
<td>OX18</td>
<td>IgG1</td>
<td>RT1 class I (several loci)</td>
<td>MHC class I+ cells</td>
<td>34</td>
</tr>
<tr>
<td>F16-4-4-11</td>
<td>IgG1</td>
<td>RT1-A</td>
<td>MHC class I+ cells</td>
<td>35</td>
</tr>
<tr>
<td>OX6</td>
<td>IgG1</td>
<td>RT1-B</td>
<td>MHC class II+ cells</td>
<td>36</td>
</tr>
<tr>
<td>OX17</td>
<td>IgG1</td>
<td>RT1-D</td>
<td>MHC class II+ cells</td>
<td>34</td>
</tr>
<tr>
<td>OX42</td>
<td>IgG2a</td>
<td>C3b receptor</td>
<td>Macrophages</td>
<td>37</td>
</tr>
<tr>
<td>3.2.3</td>
<td>IgG1</td>
<td>NKP-P1</td>
<td>All NK cells</td>
<td>38</td>
</tr>
</tbody>
</table>
90% FCS and 10% DMSO were thawed, resuspended at 2.5 × 10^6 cells/ml and the cells were incubated for 3 h at room temperature (Con A blasts) or (DuPont-New England Nuclear, Boston, MA) was added to the cell pellet, at 2 × 10^6 cells/ml. After incubation for 2 h at 37°C, nonadherent cells were removed, replated from rat long bones were resuspended at 10^7 cells/ml in tissue culture medium containing 10% FCS. Labeled cells were diluted in the same medium to 5 × 10^4 cells/ml. Threefold dilutions of effectors (100 μl) and targets (100 μl) were dispensed in triplicate into 96-well U-bottom culture dishes. Medium without effectors (100 μl) or with 1 N HCl (100 μl) was also added to triplicate wells of targets to determine spontaneous and maximal lysis, respectively. Effectors and targets in the plates were centrifuged at 65 × g for 2 min, and the plates were incubated for 4 h at 37°C and then recentrifuged in the same manner. Supernatant was harvested from each well (50 μl) and counted in a 1450 Microbeta counter (Wallac, Turku, Finland). The percent specific lysis was computed by the formula 100 × [isotope release by effectors – spontaneous release]/[maximum release – spontaneous release]. The ratio of spontaneous to maximal 51 Cr release from lysed targets was routinely <30% and averaged 16%. The SD among triplicate assays was always <5% specific lysis.

**Cell-mediated cytotoxicity assay**

Rat LN cell blast targets were generated by culture for 40 h with Con A as previously described (40). In some experiments Con A-stimulated blasts that had been kept frozen at −80°C or in liquid nitrogen at 10^7 cells/ml in 90% FCS and 10% DMSO were thawed, resuspended at 2.5 × 10^6 cells/ml in the same medium used for restimulation, and incubated for 18 h before being used. Bone marrow-derived macrophage (BMDM) targets were obtained as previously described (41). Briefly, bone marrow cells harvested from rat long bones were resuspended at 10^7 cells/ml in tissue culture medium without FCS and plated in bacteriological grade culture dishes. After incubation for 2 h at 37°C, nonadherent cells were removed, replated at 2 × 10^6 cells/ml in the complete medium supplemented with 10% FCS and 20% supernatant from L929 cells as a source of macrophage CSF, and reincubated. Cells were mechanically harvested on day 7 of culture. Infection of target cells was performed immediately before labeling, using a procedure similar to that described above for infection of stimulator cells. For labeling of target cells, 10^6 cells were centrifuged at 200 × g, the supernatant was removed by aspiration, 50 μCi of sodium [51Cr]chromate (DuPont-New England Nuclear, Boston, MA) was added to the cell pellet, and the cells were incubated for 3 h at room temperature (Con A blasts) or for 2 h at 37°C (BMDM). Labeled cells were washed three times by centrifugation at 200 × g for 7 min, counted, and resuspended in culture medium containing 10% FCS.

For cytotoxicity assays, restimulated LN cells harvested after 5 days of culture were washed and resuspended at 5 × 10^6 cells/ml in culture medium containing 10% FCS. Labeled target cells were diluted in the same

**Flow cytometric analysis of cell surface Ags**

All procedures were conducted at 4°C in PBS/2% FCS/0.01% NaN_3. Cells (5 × 10^6) were incubated with saturating concentrations of the appropriate mAb for 30 min, washed, then incubated with FITC-conjugated monoclonal goat anti-mouse IgG for 30 min. After washing, the cells were analyzed using a FACSscan flow cytometer (Becton Dickinson, Mountain View, CA).

**In vitro magnetic cell depletions**

Selected subsets of T cells were obtained after in vitro restimulation by negative selection using the following combinations of mouse mAbs, OX6, OX17, OX33, OX42, and 3.2.3, to purify all T cells plus either OX8 or OX35 to
purify CD4+ or CD8+ T cells, respectively. All procedures were conducted at 4°C in PBS/4% FCS as follows. Cells were incubated with saturating concentrations of mAb for 15 min, washed, and further incubated with goat antimouse IgG-conjugated microbeads (Dynabeads M-450, Dynal, Oslo, Norway) at a ratio of 20 microbeads/cell. Cells were washed again, resuspended in PBS-FCS, and sorted with a magnet (Dynal MPC).

**Ab inhibition assay**

Target cells were preincubated with dilutions of mAb at 4°C for 15 min in assay medium and then combined in 96-well plates as described above at an E:T cell ratio of 33. Triplicate wells were also plated containing target cells incubated with Ab at each concentration but lacking effectors. Results were expressed as the percent inhibition = [1 − (% lysis with mAb/% lysis without mAb)] × 100.

**Invasion assays**

For invasion assays, 106 blast target cells were incubated in 1 ml of culture medium without antibiotics in 24-well culture plates with live *Yersinia* at a ratio of 50 bacteria/cell in the same conditions as those used for the cell-mediated cytotoxicity assay (i.e., 2 h at 37°C followed by 1 h at 37°C with gentamicin) and washed to eliminate gentamicin. Internalized bacteria were released from infected cells by adding 1% Triton X-100 (Sigma) and measuring viable counts on agar plates. The entry of *Y. pseudotuberculosis* was expressed as the percentage of intracellular bacteria = (number of bacteria surviving gentamicin treatment/number of input bacteria) × 100.

**Light microscopy of cytospin smears**

To prepare cytospins, blast cells were incubated with live bacteria at a ratio of 50 bacterial/cell for 1 h at 37°C followed by 1 h at 37°C with gentamicin and were spun down at 72.3 × g for 8 min in a Cytospin III (Shandon, Eragny sur Oise, France). Bacteria and lymphoblasts were examined under light microscope after May-Grünwald-Giemsa staining.

**Results**

*Rats infected with Y. pseudotuberculosis mount a bacteria-specific CTL response*

To determine whether a CTL response is primed during infection with *Y. pseudotuberculosis*, LEW rats were infected intragastrically or i.p. with strains of *Y. pseudotuberculosis* harboring its virulence plasmid pYV. Rats were killed from 1–8 wk after infection, and LN cells were restimulated on irradiated *Y. pseudotuberculosis*-infected LN cells as a source of APCs. As shown, CTLs were generated after intragastric infection that efficiently killed LN blasts or BMDM targets infected in vitro with wild-type (pYV+), but not plasmid-cured (pYV−) *Y. pseudotuberculosis* (Fig. 1, A and B). Infection with either strain YPIII (Fig. 1A) or strain IP2777 (Fig. 1B) could efficiently prime rats for this specific CTL response. Furthermore, infection with *Y. pseudotuberculosis* strain IP2777 cross-primed the CTL response against the YPIII strain (Fig. 1B). In kinetics studies the CTL response peaked between 2 and 4 wk after infection (not shown). Likewise, a CTL response was generated in rats infected i.p. (not shown).

In vivo infection with *Y. pseudotuberculosis* was required to prime rat LN cells for this anti-*Yersinia* CTL response. Indeed, LN cells from uninfected rats that were restimulated in vitro on *Y. pseudotuberculosis*-infected LN cells failed to lyse *Y. pseudotuberculosis*-infected blast targets (not shown). Furthermore, following intragastric infection a significant CTL response was detectable in mesenteric LN cells but not in peripheral LN cells, indicating specificity of priming for LN cells afferent to the bacterial entry site (Fig. 1C). Finally, CTLs primed against the male H-Y minor histocompatibility Ag failed to lyse female targets infected with virulent *Y. pseudotuberculosis* (Fig. 1D).

This anti-*Yersinia* CTL response was specific for syngeneic cells infected with *Y. pseudotuberculosis*, since neither targets from another inbred strain of rats (Fig. 2A) nor targets infected with unrelated bacteria, such as *E. coli* or *S. typhimurium*, were efficiently killed (Fig. 2B).

**The *Yersinia*-specific CTL response is mediated by CD8+ T cells and is restricted by rat RT1-A**

In vitro restimulated LN cells (>90% CD2+ TCR a/b+ T cells by flow cytometry) were sorted by immunomagnetic depletion to analyze which subset of T cells was responsible for the CTL response. Sorting experiments revealed that CD8+ T cells were responsible for >90% of the CTL response (Fig. 3A). Furthermore, blocking experiments using preincubation of target cells with mAbs showed that the CTL response was dependent on rat class I, but not class II, MHC molecules (Fig. 3B). Complete blocking of the CTL response with anti-MHC class I mAbs 0X 18 (not shown) or F16-4-4-11 (Fig. 3B), the latter being specific for rat RT1-A, argues for a conventional mechanism of the CTL response.

**Target cells sensitization for killing by CTLs requires contact with viable Yersinia**

When *Y. pseudotuberculosis* used for sensitization was killed with gentamicin before contact with blast target cells, the CTL response was completely abolished (Fig. 4), indicating that interaction of blast targets with metabolically active *Yersinia* was required for further lysis by CTLs.

Possible interpretation for this result is that *Yersinia* need to invade blast targets to sensitize them for lysis. This possibility was
A CT effector cell was generated from LEW rats infected intragastrically with wild-type *Y. pseudotuberculosis* strain YPIII (pYV\(^+\)) and tested for lysis of LEW lymphblast targets infected with strain YPIII (pYV\(^-\); solid bars) or uninfected (hatched bars). Immunomagnetic sorting was performed to negatively select for CD4\(^+\) T cells and CD8\(^+\) T cells that were compared with unsorted T cells. This experiment was repeated twice with similar results.

B. CTL effectors generated as described above were tested for lysis of infected LEW lymphoblast targets that were preincubated with 2, 5, 15, or 30 \(\mu\)g/ml of anti-class I (F16-4-4-11), anti-class II (OX6), or control (\(\gamma\)C11) mAb at an E:T cell ratio of 30. Maximal percent specific lysis in this experiment was 23%. Percent inhibition = \([1 - (\%\) lysis with mAb/\% lysis without mAb]) \times 100. This experiment is representative of two.

**FIGURE 3.** Anti-*Y. pseudotuberculosis* CTL effectors are CD8\(^+\) T cells and lysis infected lymphoblast targets in a class I-restricted fashion. A, CTL effectors were generated from LEW rats infected intragastrically with wild-type *Y. pseudotuberculosis* strain YPIII (pYV\(^+\)) and tested for lysis of LEW lymphoblast targets infected with strain YPIII (pYV\(^-\); solid bars) or uninfected (hatched bars). Immunomagnetic sorting was performed to negatively select for CD4\(^+\) T cells and CD8\(^+\) T cells that were compared with unsorted T cells. This experiment was repeated twice with similar results. B, CTL effectors generated as described above were tested for lysis of infected LEW lymphoblast targets that were preincubated with 2, 5, 15, or 30 \(\mu\)g/ml of anti-class I (F16-4-4-11), anti-class II (OX6), or control (\(\gamma\)C11) mAb at an E:T cell ratio of 30. Maximal percent specific lysis in this experiment was 23%. Percent inhibition = \([1 - (\%\) lysis with mAb/\% lysis without mAb]) \times 100. This experiment is representative of two.

**FIGURE 4.** Sensitization of blast targets for cytolysis by anti-*Y. pseudotuberculosis* CTLs requires contact of target cells with live *Yersinia* and is independent of endocytosis. CTL effectors were generated from LEW rats infected intragastrically with wild-type *Y. pseudotuberculosis* strain IP2777 (pYV\(^+\); same experiment as in Fig. 1B) and tested for lysis of LEW lymphoblast targets. Targets were infected in vitro with *Y. pseudotuberculosis* strain IP2777 (pYV\(^+\); solid symbols) or uninfected (open circles). Infected targets were left untreated (solid circles) or were treated with 5 \(\mu\)g/ml CCD during infection (solid squares) or during the chromium release test (CRT; solid diamonds) or with 100 \(\mu\)g/ml gentamicin, which was added throughout target infection (solid triangles). Similar results were observed in two separate experiments.

**Table III.** Entry of *Y. pseudotuberculosis* into LEW rat lymphoblast targets

<table>
<thead>
<tr>
<th>Strains</th>
<th>% Intracellular Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>YPIII(pYV(^+))</td>
<td>0.0002 ± 0.0002(^a)</td>
</tr>
<tr>
<td>YPIIIc(pYV(^-))</td>
<td>0.008 ± 0.0003</td>
</tr>
<tr>
<td>IP2777(pYV(^+))</td>
<td>0.003 ± 0.0002</td>
</tr>
<tr>
<td>IP2777c(pYV(^-))</td>
<td>0.007 ± 0.0001</td>
</tr>
</tbody>
</table>

\(^a\) Rat lymphoblast cells (10\(^8\)) were infected in vitro with live *Yersinia* at an infection ratio of 50 bacteria/cell. After 120 min at 37°C, gentamicin (100 \(\mu\)g/ml) was added to the cells and cultures were incubated 60 min further at 37°C to kill extracellular bacteria. Blast cells were lysed by Triton X-100, and viable counts were determined. Strains are as described in Table I. This experiment was repeated twice with similar results.

\(^b\) Mean ± SD for triplicate assays.
actual antigenic targets for Yersinia-specific CTLs, that those proteins are required for efficient class I presentation of Yersinia Ags, or both.

The level of cytotoxicity against infected targets was similar whether targets were sensitized with Y. pseudotuberculosis cultured overnight at 28°C in LB medium or in conditions inducing Yops secretion in the culture medium (e.g., subculture at 37°C in Ca²⁺-deficient medium; not shown). Hence, we speculated that the secretion apparatus of Yersinia would allow effector Yops to be presented in a class I-restricted fashion by delivering such proteins directly into the target cytoplasm. To examine the contributions of several Yops encoded by Yersinia virulence plasmid to the CTL response, we tested this response against blast targets sensitized with a series of isogenic Y. pseudotuberculosis mutants selectively deficient for expression of those proteins (Figs. 6 and 8). Targets sensitized with the YP15 deletion mutant of YopH (Fig. 6) were killed at similar levels as targets infected with wild-type Y. pseudotuberculosis strain YPIII (pYV⁺; A), plasmid-cured derivative YPIIIc (pYV⁺; B), YP212 inv mutant (C), or E. coli 25922 (D). Cytospins were prepared, and the contact of bacteria (arrow) with blast cells was examined under light microscope after treatment with May-Grünwald-Giemsa stain. Magnification, ×640.

FIGURE 5. Detection of Yersinia at the surface of lymphoblast target cells. LEW Con A blast targets (10⁶/ml) were infected in vitro with Y. pseudotuberculosis at an infection ratio of 50 bacteria/cell for 2 h at 37°C followed by 1 h in the presence of gentamicin. The presence of Yersinia at the surface of infected blast cells was detected by flow cytometry using the anti-invasin mAb 3A2 (thick solid trace). The percentage refers to positive cells defined by the binding of the control mAb OKT3 (dashed trace) and the binding of mAb 3A2 at the surface of uninfected blasts (broken trace).

FIGURE 6. Invasin and virulence plasmid, but not YadA or YopH, are required for sensitization of blast targets for cytolysis by anti-Y. pseudotuberculosis CTLs. CTL effectors were generated from LEW rats infected with wild-type Y. pseudotuberculosis strain YPIII (pYV⁺) and tested for lysis of LEW lymphoblast targets infected with YPIII (pYV⁺), YPIIIc (pYV⁺), YP212 inv mutant, YPIII(pIB102) yadA mutant, or YP15 yopH mutant. Similar results were observed in two to four separate experiments for each mutant tested.

Discussion

This study demonstrates for the first time that infection of rats with one of the three Yersinia species pathogenic for humans, Y.
pseudotuberculosis, elicits a CD8+ T cell-mediated cytotoxic response. Furthermore, this response appears to depend on the RT1-A locus that is a major locus for conventional class I presentation in rats (42). Previous work suggested the possibility that such a class I-restricted T cell response would occur in the setting of infection with Yersinia (15). However, a demonstration that such a prediction actually occurred in vivo had not yet been provided.

One of the major issues regarding the possibility to mount a class I-restricted response against extracellular bacteria such as Y. pseudotuberculosis is the capacity of eukaryotic cells to process those bacteria components for class I presentation. The extracellular survival of Yersinia in the host environment is thought to result from a virulence plasmid-encoded apparatus of Yop secretion, by which Y. pseudotuberculosis, can neutralize phagocytic cells (8). Once Yersinia attach to the target cell, the secretion system is triggered, and Yops are secreted and directly injected into the eukaryotic target cytoplasm. Several of these Yops exert an action inside their target, contributing to the paralysis of phagocytosis by these cells. This system seems to compromise possible processing of Yersinia via phagocytosis by professional APCs, a mechanism important for class I presentation in the case of intracellularly living bacteria (43). However, it can lead to an alternate mechanism of class I presentation that would potentially trigger a CTL response. It is indeed likely that Yops that have access to the cytoplasm can be presented through a class I-mediated pathway (15).

In our experiments sensitization of blast target cells required contact with Yersinia that failed to invade those cells, suggesting that the whole bacteria were not penetrating the cell. The possibility that soluble proteins released from Yersinia were internalized by target cells before being processed and presented by class I molecules is unlikely, since Yersinia presentation required the living state of the bacteria, at least upon contact with the target cell, and was not blocked by addition of CCD. Furthermore, sensitizing targets with Yersinia cultured in conditions inducing Yops secretion in the culture medium did not enhance cytolysis. Our observations are consistent with involvement of the Yop-secretion apparatus. This secretion system is triggered by tight contact of Yersinia with the eukaryotic cell membrane. Indeed, inv mutants
that failed to bind to blast targets were unable to sensitize those targets either. Although other Yersinia proteins, such as YadA, can substitute for invasin in adhesion to epithelial cells and allow the Yop secretion apparatus to operate (44), this alternate pathway may not be relevant to lymphoblast cells. Indeed, the presence of YadA was not required for blast target sensitization. The absolute requirement for virulence plasmid to sensitize target cells is also consistent with the involvement of a Yop secretion apparatus. Our observation that mutants deficient in YopB or YopD failed to sensitize blast cells supports this hypothesis, since both proteins are necessary to translocate effector Yops inside eukaryotic cells. Hence, the most likely antigenic targets in our system are effector Yops. Among the effector Yops tested in our experiments, neither YopH, which was previously shown to be presented in a class I-restricted manner by eukaryotic cells (15), nor YpKpA and YopI were implicated. Interestingly, the CTL response against blast cells sensitized with the YPIII(pIB518) partial deletion mutant of YopE was retained, as opposed to its abolition observed with the YPIII(pIB522) yopE complete deletion mutant. YPIII(pIB518) mutant produces a truncated form of YopE associated with a loss of YopE function (44). Therefore, it is unlikely that YPIII(pIB518) mutant produces a truncated form of YopE asso-

References


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