H-2 RESTRICTION OF CELL-MEDIATED IMMUNITY TO AN INTRACELLULAR BACTERIUM

Effector T Cells are Specific for Listeria Antigen in Association with H-2I Region-Coded Self-Markers*

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All of the immune functions that are mediated by thymus-derived lymphocytes (T cells) are associated with cell surface structures coded by the major murine histocompatibility gene complex (H-2). Two main subgroups of T cells can be distinguished with respect to: (a) function, (b) nature of the antigen they are specific for, (c) the H-2 subregion coding for the self-cell surface marker which is involved in defining the T-cell specificity, and (d) the Ly cell surface marker. Thus, "helper" T cells reactive to chemically and/or biologically inert antigens (1), which functionally associate in as yet unexplained ways with structures coded in the I region (2-7), possess the Ly 1, but not the Ly 2 or 3 markers (8, 9). In contrast, "cytolytic" T cells, specific for viral antigens (6, 7, 10, 11), chemically reactive antigens (12), or alloantigens, (12-14) which associate functionally with the H-2K and H-2D structures, have the Ly 2,3 marker (8, 9).

Listeria monocytogenes, a facultative intracellular bacterium, triggers in mice a strong virtually exclusive cell-mediated immune response as has been classically described by Mackaness (15-17), Lane and Unanue (18), Blanden and Langman (19), and North (20). The adoptive transfer of this T-cell-mediated immunity was previously shown to be restricted by the H-2 gene complex (21). This result was the first experimental evidence for speculations of Mitchison (22) and Lawrence (23) made on delayed-type hypersensitivity to tuberculin some 20 yr ago; namely, that cell-mediated immunity was reactive to bacterial antigen only when presented on cell surfaces. Particularly the self-plus X hypothesis of Lawrence pre-empted some of the current ideas that attempt to explain the H-2 restriction of cellular immunity to viruses, intracellular bacteria, and inert, conventional antigens (23). L. monocytogenes, like viruses, fall into the category

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of infectious multiplying agents; however, they do not seem to trigger cytotoxic T cells demonstrable in vitro. Instead, *Listeria* infection sensitizes specific T cells which in vivo activate macrophages to nonspecifically increased bactericidal capacity.

In this study the effector T cells involved in *Listeria* infections were characterized with respect to the *H-2* region involved in defining the specificity. The results indicate that *Listeria*-immune effector T cells are, in addition to their specificity for *Listeria*, specific for cell surface self-structures coded in the *I* region of *H-2*. They support the hypothesis that the various *H-2*-coded self-markers are on-off switches that are operated by specific T cells for triggering differentiation of distinct, partially cell-specific functions.

Materials and Methods

**Mice.** CBA/H, BALB/c, C57BL/6, C3H/St, SJL, B10.BR, B10.A(3R), B6-*H-2*~b~ (Hz170) were purchased from The Jackson Laboratory, Bar Harbor, Maine and The Strong Foundation, San Diego, Calif. or bred at the Australian National University. C3H.OH and A.TL (24, 25) were from colonies originating from the Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Mich.

**Bacteria.** The strain of *L. monocytogenes* used and preparation of bacterial inocula have been described elsewhere (19). For intravenous (i.v.) injections the lethal dose for 50% of the animals (LD50) was approximately 5 x 10⁴ for C57BL, 2 x 10⁴ for BALB/c and CBA/H, and 2 x 10³ for A.TL and SJL. The methods for enumeration of viable bacteria in individual mouse spleens have been described by Mackaness (15-17).

**Adoptive Transfer of Antibacterial Protection.** The protocol of Mackaness was used with modifications (17). Briefly, mice were immunized i.v. with about ¼ of an LD₅₀ and sacrificed by cervical dislocation 6-7 days later. Single cell suspensions were made from their spleen cells (19). Recipients were injected usually with 5 x 10⁶ *L. monocytogenes* 2-4 h after which 3-6 x 10⁷ viable spleen cells were transferred i.v. into the infected animals. Viable bacteria were determined 24 h later (21).

**Preparative Separation of Ig-Positive and Ig-Negative Lymphocytes.** The method of Parish et al. (26) was used exactly as described. The reagents were generously provided by Dr. C. Parish, Australian National University, Canberra, Australia. Briefly, sheep IgG specific for rabbit Ig was coupled to sheep red cells (SRBC) by CrCl₄ treatment. These SRBC were then used to form rosettes which had been pretreated with hyperimmune rabbit anti-mouse Ig serum. Rosettes were separated from non-Ig-bearing cells by centrifugation through Isopaque/Ficoll. Ig-bearing cells were freed of SRBC by transient hypotonicity. After separation the Ig-positive and Ig-negative lymphocyte population was transferred in numbers that corresponded to the percentage of total spleen cells they represented before separation.

**Mixed Lymphocyte Cultures (MLC).** Primary alloreactive cytotoxic T cells were generated in vitro as described by Lafferty et al. (27). The cytotoxic activity of these MLC cells was monitored in a ⁵¹Cr-release assay as described by Cerottini and Brunner (28). The percentage of blast-like cells in the CBA/H anti-BALB/c cultures was 45% as compared to 15% in the control CBA/H anti-CBA/H cultures.

**Anti-Sera Treatment.** AKR anti-θ C3H was purchased from Bionetics, Kensington, Md. (catalogue no. 8301-01, lot no. 231-61-5). It was used at a 1:10 dilution and incubated with 5 x 10⁷ spleen cells/ml for 30 min at 4°C and for 30 min at 37°C with a selected rabbit complement (C) at a final dilution of 1:8. This treatment lysed 45% of the spleen cells specifically. The same conditions caused complete abolition of virus-specific cytotoxic activity as tested in a ⁵¹Cr-release assay in vitro (1).

**Selective Proliferation Experiments In Vivo.** 7-day immune spleen cells (1 x 10⁹) from CBA/H x C57BL/6 F₁, were transferred into parental type recipients (P) which were lethally irradiated

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Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; MLC, mixed lymphocyte cultures; P₁, P₂, parental strains of F, hybrid mice.
(850 rads) 6 h before, and dosed i.v. with $5 \times 10^9$ viable bacteria 3 h before cell transfer. The recipients were given 3 mg of ampicillin (Beecham Research Laboratories, England) intraperitoneally after 2 days. The spleens were removed on the 3rd day, and single cell suspensions were cultured (2 × 10^9 cells/100 ml) Eagle's minimal essential medium supplemented with bicarbonate, 100 μg penicillin streptomycin, and 10% fetal calf serum in 250-ml plastic Falcon tissue culture flasks) for 24 h in the presence of antibiotics to remove the majority of living bacteria (29). The surviving 50-70% of the cells were passed through cotton wool, washed in medium without antibiotics for 2 h at 37°C, and injected i.v. into parental recipients at $5 \times 10^7$ viable cells/0.4 ml.

Statistical Methods. The significance of the difference between means was assessed using Student's t test. When immune protection against Listeria is studied by using infectious doses well below the LD₅₀, the murine Listeria model is sensitive, relatively well defined, and yields reproducible results. Therefore, differences between experimental and control groups may reach statistically significant levels without representing biologically significant protection.

Results

Identity of H-2-Restricted Effector Lymphocytes. From the first report of restriction of the adoptive transfer of immunity to Listeria it was obvious that this H-2 restriction was less absolute than in the virus models and that it was more obviously cell-dose dependent. To determine whether the protection conferred by transfers of allogeneic Listeria-immune spleen cells was from T cells or non-T cells, Ig-positive and Ig-negative Listeria-immune lymphocytes were assayed for their protective potential (Fig. 1).

Ig-negative lymphocytes transferred about 2 log₁₀ of protection measured as the difference of viable counts per spleen in experimental groups as compared with control groups receiving no cells. At the doses tested the Ig-negative lymphocytes were absolutely restricted to operate optimally only in the H-2 compatible recipient. The Ig-positive cell fraction conferred a much lower degree of 0.2-0.8 log₁₀ of protection which was significant only in some combinations. However, this protective capacity was not H-2 restricted. Therefore, the Listeria-specific T-cell-mediated protection appears to be T-cell-mediated and restricted by the H-2 gene complex probably to a similar extent as has been shown for virus-specific T-cell-mediated anti-viral protection in vivo and in vitro (30, 31).

To investigate the possibility that any, i.e. also allogeneic, activated T cells could be recruited in relatively great numbers into infectious lesions and thus cause non-H-2-restricted anti-Listeria protection, MLC allogeneically stimulated lymphocytes were transferred to Listeria-infected recipients. To imitate the allogeneic donor-recipient combination, CBA/H (H-2₅) anti-BALB/c (H-2₄)-sensitized MLC cells (3 × 10⁷ per recipient) that were highly active in ⁵¹Cr-release assays were transferred to Listeria-infected C57BL/6 recipients. No significant protection was detectable. Therefore, if nonspecific T cells could have been recruited preferentially to infectious and inflammatory lesions, they were unable to act upon macrophages directly or via lymphokines to increase the latter's anti-bacterial activity. Also, anti-θ and C-treated immune spleen cells are able to transfer 0.5-0.7 log₁₀ of protection as demonstrated by Blanden and Langman (19) or North (20).

Demonstration of H-2 Restriction Depends Upon Relative Listeria-Specific T-Cell Activity of Spleen Cells. The capacity of $5 \times 10^7$ spleen cells to transfer demonstrable H-2-restricted anti-Listeria protection depends on their relative
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| Group | Listeria  |
|-------|----------|
| immune | cell donor |
| 1     | --       |
| 2     | --       |
| 3     | BALB/c   | Ig-negative (2.1 x 10^7) |
| 4     | --       | Ig-positive (4.0 x 10^7) |
| 5     | BALB/c   | Ig-positive (4.0 x 10^7) |
| 6     | --       | Ig-negative (2.1 x 10^7) |
| 7     | BALB/c   | Ig-negative (2.1 x 10^7) |
| 8     | Unseparated |
| 9     | --       |
| 10    | CBA/H    | Ig-negative (2.0 x 10^7) |
| 11    | --       |
| 12    | BALB/c   | Ig-positive (3.2 x 10^7) |
| 13    | --       |
| 14    | Unseparated |
| 15    | CBA/H    |

Transferred spleen cell population (cell numbers)

| Recipients | Log_10 Listeria per recipient spleen |
|------------|--------------------------------------|
| BALB/c     | 5.41 ± 0.20                          |
| CBA/H      | 5.67 ± 0.15                          |
| BALB/c     | 3.43 ± 0.11                          |
| CBA/H      | 5.89 ± 0.20                          |
| BALB/c     | 4.60 ± 0.04                          |
| CBA/H      | 5.22 ± 0.22                          |
| BALB/c     | 3.85 ± 0.34                          |
| CBA/H      | 3.65 ± 0.10                          |
| CBA/H      | 4.93 ± 0.13                          |
| CBA/H      | 5.59 ± 0.11                          |
| CBA/H      | 3.72 ± 0.08                          |
| BALB/c     | 4.88 ± 0.10                          |
| CBA/H      | 5.61 ± 0.19                          |
| BALB/c     | 4.87 ± 0.03                          |
| CBA/H      | 4.02 ± 0.12                          |

Log_10 Protection versus no cell control

Fig. 1. H-2 restriction of the anti-Listeria activity of surface Ig-negative lymphocytes assayed in an adoptive transfer system. Donor mice were infected with approximately 5 x 10^6 Listeria 6 day previous to cell harvest. Ig-negative spleen cells were separated from Ig-positive ones by the method of Parish et al. (26). The numbers of transferred separated spleen cells corresponded approximately to the relative numbers before separation. Recipient mice were injected with about 6 x 10^6 Listeria 2 h previous to cell transfer. Viable bacteria counts were determined 24 h later. Means ± SEM of groups of three to five mice were compared statistically: group 3 vs. groups 7 or 8: not significant (NS); 3 vs. 1, 5, 10, 12, 14: P < 0.001; 4 vs. 6: NS; 4 vs. 9: P < 0.01; 10 vs. 12, 14: P < 0.01; 1 vs. 12; NS; 1 vs. 14: P < 0.05; 11 vs. 2, 4, 6, 9, 13: P < 0.001; 11 vs. 15: NS.

specific activity. In Table I, one example of many, spleen cells of CBA/H-immune donors which are poorly active in syngeneic recipients transfer about the same level of protection to allogeneic recipients. BALB/c-immune spleen cells of great syngeneic activity, transfer to allogeneic CBA/H again the same background protection of about 0.6-1.0 log_{10} (Table I). Thus, under standard experimental conditions only spleen cell populations that confer greater than 1 log_{10} of protection will allow specific H-2 restriction to be measured reliably.

Genetic Mapping of the H-2 Regions Involved in Defining T-Cell Specificity. Three sets of experiments attempted to evaluate whether compatibility at: (a) the D or K region of H-2 alone, or (b) the I region alone was sufficient to transfer maximal protection, and (c) whether wild-type H-2K^a Listeria-immune spleen cells were able to transfer protection to H-2K'^a or H-2K'^b mutant mice.

Tables II and III and Fig. 2 indicate that H-2 compatibility between donors and recipients at the K end results in much more efficient transfer of protection than if the D end is shared. B10.A-immune spleen cells protect CBA/H recipients and B10.BR lymphocytes protect B10.A recipients each by more than 3 log_{10} (P < 0.00001) as efficiently as fully syngeneic combinations (Table II, Fig. 2).
Table I

Relative Independence from Specific Anti-Listeria Activity of Non-H-2-Restricted Background Protection by Unseparated Immune Spleen Cells

| Donors of immune* spleen cells | Log$_{10}$ Listeria per CBA/H spleen | Protection vs. control | Log$_{10}$ Listeria per BALB/c spleen | Protection vs. control |
|--------------------------------|--------------------------------------|------------------------|---------------------------------------|------------------------|
| Listeria immune                |                                      |                        |                                       |                        |
| CBA/H                          | 4.95 ± 0.23§†                        | 1.09                   | 5.10 ± 0.15                           | 0.76                   |
| BALB/c                         | 5.09 ± 0.33                          | 0.95                   | 3.47 ± 0.23§                         | 2.39                   |
| Normal CBA/H                   | 5.75 ± 0.15                          | 0.29                   | 5.65 ± 0.10                           | 0.21                   |
| Normal BALB/c                  | 5.79 ± 0.17                          | 0.25                   | 5.58 ± 0.16                           | 0.26                   |
| Control (no cells)             | 6.04 ± 0.12                          |                        | 5.85 ± 0.15                           |                        |

* Donor mice were infected with about 3 × 10$^6$ Listeria 6 days previous to assay.
† Viable counts were determined 24 h after infection with approximately 6.5 × 10$^3$ Listeria and 22 h after adoptive transfer of 5 × 10$^5$ spleen cells.
§ Mean ± SEM of viable spleen counts of groups of five mice. Significance vs. normal or no cell controls.
|| P < 0.05.
¶ P < 0.001.

Table II

Adoptive Transfer of Listeria Immunity in Various Mouse Strain Combinations

| Spleen Cell* donor | Log$_{10}$ viable Listeria per spleen of recipient mice |
|--------------------|--------------------------------------------------------|
|                    | H-2 | I | K | S | D | H-2 | I | K | S | D |
|                    | CBA/H | k | k | k | k | B10.D2 | d | d | d | d | B10.A | k | k | d | d | C3H.OL | d | d | k | k |
| B10.BR             | k | kkk | k | k | 2.41 ± 0.18§† | 4.85 ± 0.17* | 2.10 ± 0.09‖ | 3.87 ± 0.09** |
| B10.D2             | k | kkk | d | d | 1.99 ± 0.06§ | 3.67 ± 0.13** | 2.20 ± 0.16‖ | 3.51 ± 0.13** |
| B10.A              | k | kkd | d | d | 5.07 ± 0.14§ | 2.67 ± 0.13‖†† | 4.02 ± 0.16** | NT |
| No cell controls   | k | kkk | d | d | 5.30 ± 0.17 | 5.32 ± 0.03 | 5.61 ± 0.06 | 4.88 ± 0.08 |

* Spleen cell donors were infected with 9.5 × 10$^6$ L. monocytogenes 7 days previous to transfer. 5 × 10$^5$ spleen cells were transferred to recipients infected with 5 × 10$^6$ Listeria 2 h previously.
† See Materials and Methods. Counts were determined 24 h after transfer.
§ Mean ± SEM of groups of three to five mice. Statistical significance of protection as compared with no cell controls.
|| P < 0.00001.
‖ Not significant.
** P < 0.001.
†† Significantly smaller than B10.A, P < 0.001.

B10.A and B10.D2 protect each other considerably less by about 1.5 log$_{10}$ (P < 0.001). The lesser importance of D-end determinants contributing to protection in this model was further emphasized by the fact that B10.BR cells conferred much less protection (i.e., 1.1 log$_{10}$) to C3H.OL recipients as compared with the 3.5 log$_{10}$ protection transferred to B10.A (Table II, Fig. 2). Experiment 1 in Table III suggests that in reactions traced to compatibility at the K end, the I-A
subregion and K region seem to be crucial. The protective potentials of the two (CBA/H and C57BL/6) spleen cell populations studied are comparable; both protect syngeneic recipients by about 3.1 log₁₀. However, CBA/H-immune cells conferred about 2.3 log₁₀ protection to K plus I-A compatible recipients as compared with 1.4 log₁₀ transferred by I-B, I-C, S, and D compatible C57BL/6 immune cells. Experiment 2 further suggests that the I-A subregion may be responsible for most of the protective effect, since A.TL-immune spleen cells protected H-2K mutant SJL mice markedly less than I plus S region compatible CBA/H mice. Immune T cells from mice possessing the wild type K, I-A, and I-B regions protected H-2K⁺ mutant mice which share the I region with wild-type mice, as well as they protected wild-type recipients (Table IV). These results, unlike those involving virus-specific cytotoxicity in which H-2K⁺(H-1)-immune spleen cells cannot protect wild-type H-2K⁺ recipients in vivo or efficiently lyse such cells in vitro or vice versa (30–32), suggest that compatibility at the I region is sufficient for adoptive transfer of immunologic protection against Listeria.

Attempts to Separate T-Cell Specificities of F₁ Hybrid Mice by Selective Proliferation. With an experimental approach that was originally applied in the lymphocytic choriomeningitis virus (LCMV) system to dissect cytotoxic T-cell specificities in F₁ hybrids (11, 33), several attempts were undertaken to evaluate whether the same F₁ T cells could protect recipients of both P strains or whether two distinct T-cell populations were specific, one for P1 and the other for P2.

Irradiated parental recipients were infected with Listeria and transfused with
FIG. 2. Adoptively transferred anti-

Listeria protection expressed as the difference of viable bacteria counts in spleens of control mice receiving no cells minus the counts in spleens of mice transfused with 7-day immune spleen cells of various H-2 types. From Table II.

F1 7-day immune spleen cells. Irradiation prevents macrophage to proliferate in the bone marrow and to reach the infectious lesion. Therefore, Listeria grew rapidly. Antibiotic treatment in vivo after 2 days and after spleen cell removal at 3 days followed by cultivation of spleen cells in medium containing antibiotics for 24 h eliminated bacteria successfully. These spleen cells were then assayed for their protective potential in the final recipients by using the standard protocol. These experiments were difficult to perform and many intermediate recipient mice died of the overwhelming infection. Unfortunately, therefore, the double transfers could not be made in all reciprocal permutations, F1 → P1 → P1, F1 → P2 → P2, F1 → P1 → P2, and F1 → P2 → P2. Nevertheless, in two successful experiments out of four attempts the immune F1 cells transferred through P1 were subsequently able to protect P1 recipients better than P2 recipients (Table V). The effector cells assessed in these double transfer experiments were T cells as shown by their susceptibility to anti-θ treatment (Table V, exp. 2). The two examples to suggest that F1 possess two separate Listeria-immune T-cell specificities, each specific for one parental H-2 haplotype.

Discussion

Analysis of the previously described finding (21) that Listeria-immune T cells are H-2 specific in their capacity to transfer immunity adoptively to naive
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Anti-Listeria Protection of Spleen Cells of Immune H-2Kb Wild-type Mice in H-2Kb Mutant Mice

| Donor | H-2 | Recipient | Log₁₀ Listeria per spleen$ | Protection |
|-------|-----|-----------|-----------------------------|------------|
|       | I-A | I-B | I-C | I-A | I-B | I-C |                      |            |
| B10.  | b   | b   | b   | b   | b   | b   | 2.92 ± 0.15          | 2.12       |
| B10.A(3R) | b | bbd | d   | d   | B6-H-2** | b   | b   | 3.27 ± 0.14          | 1.77       |
|       | k   | k   | k   | k   | B6-H-2*  | b   | b   | 4.65 ± 0.06**        | 0.39       |
| 0     | b   | b   | b   | b   | B6-H-2  | b   | b   | 5.04 ± 0.12          |            |
| B10.  | b   | bbd | b   | b   | B6-H-2  | b   | b   | 3.21 ± 0.08          | 1.85       |
| B10.A(3R) | b | bbd | d   | d   | B6-H-2  | b   | b   | 3.49 ± 0.24          | 1.17       |
| CBA/H | k   | b   | k   | k   | B6-H-2  | b   | b   | 4.69 ± 0.041         | 0.37       |
| 0     | b   | b   | b   | b   | C57BL/6 | b   | b   | 3.12 ± 0.11          | 1.75       |
| B10.  | b   | bbd | d   | d   | C57BL/6 | b   | b   | 3.44 ± 0.06          | 1.34       |
| CBA/H | k   | b   | k   | k   | C57BL/6 | b   | b   | 4.39 ± 0.07**        | 0.48       |
| 0     | b   | b   | b   | b   | C57BL/6 | b   | b   | 4.67 ± 0.16          |            |
| CBA/H | k   | k   | k   | k   | CBA/H   | k   | k   | 3.87 ± 0.02          | 1.35       |
| 0     | k   | k   | k   | k   | CBA/H   | k   | k   | 5.25 ± 0.09          |            |

* Donor mice were infected with 1 × 10⁴ Listeria 7 days previous to transfer.
† Recipients were transfused with 5 × 10⁷ spleen cells 2 h after infection with 4.2 × 10⁷ L. monocytogenes. Determination of viable counts 24 h after transfer, see Materials and Methods.
§ Means ± SEM of groups of three to five mice. Protection was significant when compared with control mice not given cell transfer (first symbol) or when compared with recipients of allogeneic cells (second symbol).
||P < 0.001.
¶ P < 0.01.
** Not significant.
†† P < 0.05.

Protective Effect of Listeria-Immune F₁ Spleen Cells Which Were Selected In Vivo by Transfer for 3 Days to Infected P₁ Recipients and Thereafter Assayed In P₁ or P₂ Final Recipients for Anti-Listeria Protection*

| Original donor (H-2 type) | Intermediate recipient (H-2 type) (350 rad) | Cell treatment | Final recipient (H-2 type) | Log₁₀ viable Listeria in spleen 48 h after cell transfer |
|---------------------------|---------------------------------------------|----------------|----------------------------|----------------------------------------------------------|
| Exp. 1                    |                                             |                |                            |                                                          |
| CBA/H × C57BL/6 F₁ (k × b)| CBA/H (k)                                   |                | CBA/H (k)                  | 3.40 ± 0.12||                                               |
| None                      | None                                        |                | CBA/H (k)                  | 4.46 ± 0.10                                               |
| Exp. 2                    |                                             |                |                            |                                                          |
| CBA/H × BALB/c F₁ (k × d)| CBA/H (k)                                   |                | CBA/H (k)                  | 2.83 ± 0.07                                               |
| None                      | None                                        |                | CBA/H (k)                  | 5.65 ± 0.07                                               |
| CBA/H × BALB/c F₁ (k × d)| CBA/H (k) (2.5 × 10⁶) AKR anti-O + C’       |                | CBA/H (k)                  | 5.23 ± 0.11                                               |
|                           | CBA/H (k) (2.5 × 10⁶) Nor AKR serum + C’   |                | CBA/H (k)                  | 3.73 ± 0.17                                               |

* For experimental details see Materials and Methods.
† Final recipient mice were infected with about 5.1 × 10⁴ (exp. 1) and 4.0 × 10⁴ (exp. 2) Listeria 2 h previous to cell transfer. Viable counts were determined 48 h later.
§ Means ± SEM of three to four recipients.
||P < 0.001.
¶ P < 0.01.
** Not significant.
†† P < 0.05.

recipients revealed that, first, the H-2 restriction of effector T cells is virtually absolute under the conditions employed; second, the self-marker recognized is probably coded by the I-A subregion of H-2; third, in F₁ heterozygotes at least two separable T-cell specificities exist—one each for Listeria antigen in associa-
tion with the two respective parental H-2 haplotypes. Thus, the H-2-associated specificities of effector T cells generated during virus infections and infections with intracellular bacteria differ with respect to the H-2 subregions involved (6, 7); the effector activity of Listeria-immune spleen cells is specific for an I-region-coded structure, which is analogous to the situation for helper T cells (2, 3), in contrast to the specificity of cytolytic T cells which exhibit specificity for K or D structures.

The selective proliferation experiments in F1 hybrids suggest strongly that the Listeria-immune T cells are H-2I haplotype specific. Such T cells appear to be specific either for a structural complex of self plus Listeria antigen or for bacterial antigen that is expressed on the cell surface and which is modified by I-region-linked enzymes (34) (altered-self, Fig. 3). Alternatively, T cells may possess two independently and clonally expressed receptors, one for Listeria antigen and one for the I-region-coded self-structure (dual recognition); the clonality of the expression of the self-recognition structure is suggested by the F1 experiment (11, 35–37). If one uses the dual recognition model to explain H-2 restriction, the haplotype specificity of Listeria-immune T cells, suggests allelic exclusion of recognition structures for I-coded self-markers. Since I-coded self-markers are codominantly expressed on B cells (38), and possibly also on macrophages, allelic exclusion of I recognition makes a mutual like-like self-interaction model of I-coded structures an unlikely model to explain H-2 restriction (2, 6). Therefore, if the findings from virus-specific cytotoxicity models can be translated to noncytotoxic I-region-specific T-cell activity, it is more likely that I restriction reflects unidirectional recognition by T cells of I-coded self-markers on target cells (35–39). However, as has been discussed extensively for the T helper and the virus models, the available data do not allow one, at the moment, to distinguish between the two basic models of T-cell recognition: dual recognition or altered-self (2–7, 34–39).

How can the differential association of various infectious agents with K, D, or I-coded structures be explained? For the following discussion we make the assumptions that, first, the mammalian-immune system is the vertebrate's solution to combat intracellular infectious agents and, second, that cell-mediated immunity is the ancient part of the immune system. The relative importance of antibodies and T cells in immunity to infection by intracellular bacteria or viruses in primary infections need not be discussed here. The current concepts have been reviewed recently (40–43). Strong evidence exists in several murine infectious disease models that specific effector T cells are generated very rapidly after infection, i.e., within 2–4 days; for example, in Listeria (25), pox virus (40) or LCMV (R. M. Zinkernagel, unpublished data) infections. Since any means of eliminating or decreasing the functional T-cell compartment in mice results in a drastic failure to control virus or intracellular bacterial growth during this 2- to 4-day period after infection, the crucial role of T cells in controlling primary infections very early and rapidly is strongly indicated. Nevertheless, one cannot fully exclude contributions by pre-existent antibodies that are specific or non-specific, i.e. cross-reacting, or specific antibodies generated with T-cell help. Immunity in the Listeria model is extreme in its apparent independence from antibodies, whereas protection against other intracellular bacteria like Salmo-
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Can the results presented here contribute to a better understanding of effector T-cell specificity and a possible mechanistic role of I and K or D coded self-markers? The crucial functional antigenic characteristic of viruses and intracellular bacteria is that they "hide" behind the cell walls and can propagate themselves more or less directly from cell to cell avoiding the circulation and extracellular spaces where they could be subject to attack by antibody plus C. However, two important points are worth stressing: first, this hiding-away is not totally successful since after viral infection, and probably also after intracellular bacterial infection, foreign specific antigenic determinants are expressed on the infected cells; and second, viruses and bacteria differ in their habitats. Thus, with differential preferences called "tropisms," viruses can actively infect phagocytic or nonphagocytic cells because these cells have the relevant cell surface structures allowing viruses to absorb to and penetrate actively into the passive cells. No such mechanism exists for intracellular bacteria, which, in contrast, have to be actively phagocytized by cells; this, in turn, limits their habitat to phagocytic cells.

If T cells must recognize self-markers in addition to foreign antigens on cell surfaces to become sensitized or to mediate effector function, it seems necessary that the self-marker involved in cell-mediated immunity against viruses be an ubiquitous one. Major transplantation antigens coded in the K and D regions of H-2 exist in varying amounts on virtually all cell surfaces of an individual. In contrast, the self-marker involved in cellular immunity to intracellular bacteria may be largely restricted to phagocytic cells.

T-cell-mediated protection against virus infection can occur in several ways; the relative importance of one or the other depends upon several factors, particularly the pathogenesis of the virus disease in question and the time after infection. Two possible main effector mechanisms are: first, virus that infects cells causes cell surface changes very shortly after penetration, i.e., before infectious virus progeny are assembled (31, 32, 47, 48). Recent evidence has shown that cytotoxic T cells inhibit the amounts of virus produced by target cells actively infected with vaccinia virus, and that the degree of inhibition directly correlates with the cytolysis activity of such T cells; once infectious progeny are assembled, T cells cannot directly influence viral titers (47). These findings indicate that the rapidly generated cytotoxic T cells can therefore control virus

\[ \text{Fig. 3. Models for T-cell recognition of } Listeria \text{ antigen on macrophage surfaces.} \]
Fig. 4. Models of T-cell-mediated macrophage activation. Although only the dual recognition model for T-cell recognition is depicted, the model is also applicable to the single recognition model. In both, antigenic specificity is given by the Listeria (1)-specific part of recognition and the activation signal is delivered through the I-region-coded self-structure (2). This could result in direct macrophage activation resulting in digestion of bacteria. Alternatively, the recognition of Listeria antigen plus I-coded self-marker could result in lymphokine production (3). Such factors could then trigger the activation (4) of surrounding (5) macrophages.

production and spread by directly destroying infected cells via a cytolytic signal before infectious progeny are assembled. Second, T cells may act in a specific anti-viral manner or by nonspecific mechanisms such as release of immune interferon or by lymphocyte-mediated recruitment and activation of macrophages to exert increased virucidal activity. Alternatively, specific T cells may "activate" macrophages thereby exposing the relevant antigens to increased virucidal activity by direct contact, but via a noncytolytic signal.

Similarly, it is generally agreed that T-cell-mediated protection against intracellular bacteria occurs by mechanisms similar to the latter model (43). Accordingly, T cells are either triggered specifically to release lymphokines which nonspecifically recruit and activate macrophages to increased bactericidal capacity and/or alternatively, as proposed here, may activate macrophages expressing the relevant foreign and self-surface antigens by direct contact (Fig. 4).

So far no cytotoxic effector T cells has been demonstrated in immunity to Listeria. This is obviously no strong argument against their existence, and their absence may be explained by technical difficulties in obtaining and maintaining Listeria-infected macrophage target cells even for limited periods of time. However, there are good theoretical reasons why cytolytic cell-mediated immunological interactions may not be crucial in dealing with intracellular bacteria. In contrast to viruses, intracellular bacteria (comparable to any inert conventional antigen or toxins) never lose their anatomical and functional identity. (As discussed previously, for a brief period after penetration during the eclipse phase, viruses do not exist as individual infectious entities and, therefore, are vulnerable to destruction by host cell lysis.) In the case of bacteria, therefore, lysing the host cell never kills the organism, but only results in release of intact bacteria. Thus, growth and spreading of Listeria is controlled only when phagocytic cells have acquired the capacity to digest the bacteria efficiently. T cells which recognize antigen in association with I-coded structures may send through this I structure a noncytolytic, but "activating," signal to the recognized phagocytic cell. Alternatively, this I-associated T-cell recognition may trigger the T cell itself to release lymphokines, which, in turn, could activate macrophages.
Thus *Listeria*, a biologically active, infectious agent, elicits a T-cell response that has the I region-associated specificity characteristics of T cells handling chemically inert or noninfectious antigens and differs from the H-2K- and D-restricted murine virus models. Based on the considerations discussed above, it now appears that K and D regions code for structures where cytolytic signals can be delivered most efficiently by T cells; in contrast, as suggested previously (2), the I region codes for structures where predominantly noncytolytic T-cell signals that trigger cell-specific differentiation processes can be delivered. Thus, antigen-specific triggering of a certain I-coded structure on macrophages may, for example, result in differentiation of its digestive enzymes, whereas on certain B cells such a signal via another I-coded structure may result in the switch from IgM to IgG production.

Summary

The protective activity of anti-*Listeria*-immune T cells assayed in an adoptive transfer system is H-2 restricted. As shown in the present studies, the demonstration of the restriction is directly dependent on the dose and the relative protective activity of spleen cells. In addition, some H-2-unrestricted protection is conferred predominantly by other than immunoglobulin-negative spleen cells. Thus, the activity of *Listeria*-immune T cells appears to be "absolutely" restricted and is in this respect comparable to in vivo T-cell-mediated anti-viral protection. The predominant genetic region of H-2 coding for the structures which are mainly involved in this restriction in T-cell immunity to this prototype intracellular bacterium is the I region. The specificity of *Listeria*-immune T cells is determined by the H-2 haplotype of the donor. Thus, F1 hybrids seem to possess at least two separable sets of T cells, each specific for one parental haplotype. As is true in the virus model, the results cannot distinguish between an altered-self or a dual recognition model of T-cell recognition to explain H-2 restriction. They are, however, compatible with the idea that I-coded cell surface structures may serve as receptors for cell-specific differentiation signals, which trigger direct or lymphokine-mediated activation of macrophages to manifest increased bactericidal capacity. The interesting parallels in self-marker recognition by T cells in the virus and intracellular bacterium systems, respectively, appear to be reasonably explained by the different types of signals transmitted by T cells to various target cells via the distinctly different self-markers employed (i.e., K or D vs. I).

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