fected with the Gilliam strain were died. And the infected athymic mice treated with T cells from non-immune normal mice were also taken ill and died. On the other hand, the athymic mice infected with the low virulent Irie strain were survived by the treatment with both immune and non-immune mouse T cells. The serum antibodies were recognized from the survived mice.

It was suggested that the progress of rickettsial infection was protected by construction of cellular immune system.

Moreover, appearance and duration of protective effect of T cells from mice after immunization were observed. T cells from mice immunized 7 and 10 days after with the Gilliam strain conferred no protection against the mice infected with the homologous strain. At 14 day post-immunization, the infected mice became ill, but all of the normal mice and a part of the athymic mice were survived. Practically complete protection against lethal infection was observed from days 21 for normal mice and 28 for athymic mice to more than month 12.

5. Effect of anti-Thy-1, anti-Lyt-1 and anti-Lyt-2 alloantiserum treatment against immune mouse T cells. In the BALB/c mouse strain, codes for lymphocytes cell membrane alloantigenic determinants are Thy-1.2, Lyt-1.2 and Lyt-2.2. Then, the immune mouse T cells were treated with anti-Thy-1.2, anti-Lyt-1.2 or anti-Lyt-2.2 alloantiserum for 60 min. at 4°C respectively, following that the T cells were treated with rabbit complement for 60 min. at 37°C. These immune T cells treated with the alloantiserum and complement were transferred to athymic mice infected with Gilliam strain. The infected athymic mice transferred immune T cells treated with anti-Thy-1 or anti-Lyt-1 alloantiserum showed pathological symptoms and died at the same time as the control mice which did not receive immune mouse T cells. On the other hand, the transfer of immune mouse T cells treated with anti-Lyt-2 alloantiserum gave the mice a longer existence for a few days, but the mice were taken ill and finally died.

These findings showed that inactivation of T cells meant destruction of immunological defensive mechanism in the rickettsial infection. Particularly, helper T cells sensitive to anti-Lyt-1 alloantiserum seem to play the most important role, but the function was restricted without suppressor or cytotoxic T cells sensitive to anti-Lyt-2 alloantiserum.

6. Effect of transfer of immune mouse serum to infected mice. Immune mouse serum against Gilliam strain was transferred intraperitoneally twice to athymic and normal mice at the same time as infection (0.5 ml) and 7 days after infection (0.2 ml). The normal mice became ill, but survived. On the other hand, the athymic mice were died.

CONCLUSION

It may be concluded that inhibition of progress of tsutsugamushi disease was principally due to cellular immune mechanisms and production of serum antibody was thymus dependency.
cavity of Listeria-treated mice, and to examine the bactericidal activity of these macrophage subsets.

**MATERIALS AND METHODS**

**Bacterium.** Listeria monocytogenes, serotype 4b, was originally obtained from a fresh human isolate. The LD₅₀ by intraperitoneal route of infection in Swiss mice was approximately 2 x 10⁴ viable bacteria.

Peritoneal cells from female Swiss mice weighing about 20 g were harvested after intraperitoneal injection with viable bacteria at various stages. Activated macrophages were produced according to a two-step immunization. Mice were first immunized by i.p. injection of sublethal dose of 2 x 10⁴ viable bacteria. After 7 days, the cells were elicited by i.p. injection of 10⁷ viable listeria.

**Cytochemistry.** Macrophages were examined for the demonstration of peroxidase activity by the method of Fahimi (1969) and catalase activity according to Novikoff (1972). Superoxide anion and H₂O₂ generation was demonstrated by the methods of Karnovsky et al.

**Peritoneal macrophages** were differentiated by the criteria of van der Rhee (1979). The intracellular killing activity of peritoneal macrophages were evaluated by Armstrong and Hart (1971).

**RESULTS**

In the peritoneal cavity of Listeria-treated mice, the existence of five types of macrophages was shown. These macrophage subsets were distinguished by localization of peroxidase activity and by the presence of characteristic types of granules. Resident macrophages were characterized by peroxidase reaction product in rough surfaced endoplasmic reticulum and nuclear envelopes. Exudate monocytes were identified by the presence of peroxidase positive primary granules and peroxidase negative secondary granules (Daems & Brederroo 1973, Ogawa 1978). The appearance of macrophage granules in all immature macrophages and mature macrophages made them quite characteristic (van der Rhee 1979). Immature macrophage contained both peroxidase positive granules and macrophage granules. Mature macrophage had only macrophage granules. The large epitheloid cells satisfied by the criteria of van der Rhee were also found.

There was a strong increase in the number of exudate monocytes from 3 hours to 16 hours after infection. A significant increase in the number of immature macrophages was found from 16 hours to 4 days. The number of mature macrophages was gradually elevated from 72 hours until 7 days. There was decrease in the number of resident macrophages from 3 hours. It returned to normal 4 days after infection. A small number of epitheloid cells could be found from 4 days.

Elicitation of mice with two-step immunization produced an increase in the number of immature macrophages and exudate monocytes. In immature macrophages and exudate monocytes, the number of damaged bacteria was significantly higher than that of intact bacteria. Evidence was frequently seen of fusion having occurred between peroxidase positive granules and bacteria containing phagosomes. Sign of fusion were infrequently if the bacteria were intact, but almost universal if damaged. On the contrary, in mature macrophages epitheloid cells and resident macrophages most bacteria were intact. Superoxide anion and H₂O₂ generation in mature macrophages, epitheloid cells and resident macrophages were quantitatively less than those found in immature macrophages and exudate monocytes. Immature macrophages and exudate monocytes displayed significantly greater listericidal activity than mature macrophages, epitheloid cells and resident macrophages did.

**DISCUSSION**

An early indication of the functional heterogeneity of macrophages with respect to antibacterial activity was derived from in vitro studies showing that although some bacteria are killed after phagocytosis, other survive and multiply.

The data presented in this report document significant differences among mouse peritoneal macrophage subsets with regard to listericidal activity. These observations provided the explanation that the host’s defence was induced by prompt influx and purposeful development of monocyte-derived macrophages in the center of infection.

REFERENCES


(6) Use of Pseudomonas Aeruginosa Vaccine in Patients with Severe Airway and Transit Zone Infection

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The patients with chronic respiratory infection will have an increased incidence of Pseudomonas infections. Respiratory infection in diffuse panbronchiolitis is frequently severe and follows a smoldering course punctuated by acute exacerbations of bronchitis or pneumonia baseline of chronic productive cough and bacterial colonization. Pseudomonas organisms are seldom eradicated from the sputa, regardless of the antibiotic therapy regimen. Because antibiotics have not solved Pseudomonas infection, the use of immune prophylaxis against Pseudomonas remains relevant. Active immunization of susceptible patients with Pseudomonas vaccine has been advocated as a possible means of preventing or improving the prognosis of Pseudomonas infection.

MATERIALS AND METHODS

Between 1972 and 1980, 21 patients hospitalized with chronic respiratory infection were given Pseudomonas vaccines. Patients consisted of 19 diffuse panbronchiolitis and 2 bronchiectasis, using multi component vaccines prepared from a common antigen (OEP), protease, elastase and exotoxin toxoids of Pseudomonas aeruginosa by Homma.

Course of vaccination was defined as subdermal injections of vaccines, given twice a month.

Patients received doses on a schedule of 10 mcg, if side effects did not occur, necessitating use of larger doses 50 mcg.

Antibody-titer for Pseudomonas aeruginosa in the serum has been measured by passive hemagglutination test using OEP (original endotoxin protein-Homma), elastase, protease as coated antigen.

RESULTS

A total of 21 patients consisting of 19 with diffuse panbronchiolitis and 2 with bronchiectasis were vaccinated. Of 10 patients with Pseudomonas infection after vaccination, 4 patients died and 3 patients discontinued vaccination. The survival was significantly longer in the vaccinated group than in control patients.

Of 11 patients with no history of Pseudomonas infection, after vaccination 5 patients had Pseudomonas infection. Vaccination resulted in no significant prophylactic effect in patients with no history of Pseudomonas infection. Serum OEP-HA, elastase-HA, protease-HA antibody titers rose well above the titers before immunization in 7 patients after vaccination.

The Pseudomonas vaccines elicited adverse reaction in some patients. Acute anaphylaxis did not occur in any patient. Most reactions consisted of local pain and induration at the injection site.

The results of this study suggest that vaccination will result in an increase probability of survival.