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Scintigraphic Diagnosis of Syphilitic Lesions in Rabbits by Radiolabelled Monoclonal Antibodies Specific for *Treponema Pallidum*

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Immunoscintigraphy with radiolabelled monoclonal antibodies has been widely used to detect solid tumours. The purpose of this study was to investigate its potential for the specific localization of syphilitic lesions. F(ab')$_2$ fragments were prepared from murine monoclonal antibodies against *Treponema pallidum* produced in our laboratory and labelled with ^{131}I. Bilateral testicular infections were created in rabbits by inoculation with *T. pallidum* or *Staphylococcus aureus*. Seven to 10 days after inoculation, radiolabelled antibodies were injected intravenously. Serial gamma images were then taken 2 h after the injection and at 24 h intervals thereafter. Beginning as early as 2 h post-injection, the testicles could be visualized with either specific or non-specific antibodies. Gamma images in the monoclonal antibody-treated, *T. pallidum*-infected group persisted up to 48 h post-injection. Lesions were not discernible from background in the *S. aureus*-infected group injected with the monoclonal antibody and the *S. aureus*-infected and *T. pallidum*-infected groups injected with the polyclonal antibody at 24 h post-injection or later. Therefore, due to its ability to differentiate between specific and non-specific antibody-generated images from 24 h post-injection, immunoscintigraphy using monoclonal antibodies specific for *T. pallidum* may be employed as one of the methods to diagnose difficult cases of syphilitic internal organ involvement as well as syphilis infection in seronegative HIV-infected patients. *Key word: Immunoscintigraphy.*

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Since the report of tumour localization by radiolabelled antibodies directed against tumour antigens in 1953 (1), the in vivo delineation of tumour masses by the technique of "specific imaging" has received increasing attention (2). Polyclonal or monoclonal antibodies directed against tumour-associated or other antigens (3–5), when labelled with either radioiodine or indium-111 (^{111}In), will localize in specific sites and can be detected by external scintigraphy with the conventional gamma cameras. In addition to imaging tumours or vascular diseases, the technique has recently been used for the specific localization of certain types of infections (6, 7). Rubin et al. (7) reported that radiolabelled murine monoclonal antibodies directed against Fisher immunotype 1 *Pseudomonas aeruginosa* could be used to detect sites of infection with *P. aeruginosa*.

Although syphilis can usually be diagnosed with serologic tests for syphilis, these tests are not useful in detecting syphilitic internal organ involvement, from which it is difficult to detect *Treponema pallidum*. Thus a specific and non-invasive clinical tests that can be performed to assess the presence and extent of syphilitic lesions is desirable.

To assess the potential of immunoscintigraphy for specific targeting of syphilitic lesions, we have utilized a rabbit model with testicular *T. pallidum* infection and have detected syphilitic lesions with a radiolabelled *T. pallidum*-specific monoclonal antibody.

MATERIALS AND METHODS

Microorganisms

T. pallidum, Nichols strain, from the Center for Disease Control, Atlanta, was used throughout the study. *T. pallidum* was purified from rabbit testicular tissue by Percoll density gradient centrifugation (7–10 days after inoculation into rabbit testicles) and 1 ml of $2–3 \times 10^7$ treponemes/ml was inoculated into both rabbit testicles to induce an adequate inflammatory reaction. Negative control groups were injected with 1 ml of suspension containing 5×10^8 *S. aureus*. Immunoscintigraphy was performed 7–10 days after the inoculation, when the testicles were hardened and indurated by the inflammatory reaction. The *S. aureus* (ATCC 25923) was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.).

Rabbits

Specific pathogen-free, 64 male New Zealand white rabbits weighing 2.5–3.0 kg were used for the experiments. They were housed for 2 weeks on standard diet before the experiments, and *T. pallidum* or *S. aureus* infection was produced in 32 rabbits each. Each group was subdivided into two groups, into which monoclonal antibody (MAb) and polyclonal antibody (PAb) were injected.

Monoclonal antibody production

MAb was made by immunizing BALB/C mice with *T. pallidum*. Their spleen cells were fused with SP2/0 or V653 myeloma cells and the clones secreting IgM, IgG and IgA antibodies were screened. Their isotypes were determined using a mouse monoclonal isotyping kit (Hyclone Lab., Utah, U.S.A.). Among the MAbs produced, an antibody against the 47 kDa *T. pallidum* protein, YS-307 (IgG2a), was selected due to its high specificity and immunoreactivity as determined by ELISA. For the mass production of MAb, pristane (2, 6, 10, 14-tetramethyl pentadecane; Sigma Chem. Co., St. Louis, MO, U.S.A.) pretreated BALB/C mice were given YS-307 clone cells intraperitoneally. Ascitic fluid was collected 7–14 days later, centrifuged and the supernatant was collected (8).

Monoclonal antibody purification

A protein A-Sepharose CL-4B column, 1.0 cm × 10 cm, was used for the purification of MAb. Ascitic fluid was diluted with 15 ml of Tris buffer (pH 8.6) and passed through the column, and tubes showing an optical density value of more than 0.1 by ELISA were collected and concentrated to 1.5 mg/ml using Centriprep-10 (Amikon Division, Danvers, MA, U.S.A.) (8).

Fragmentation of IgG to F(ab')$_2$

IgG was digested into F(ab')$_2$ with pepsin at 37°C, pH 4.1 and pH 4.5. At the pepsin concentration of 0.1 mg/ml, incubation times of 2, 4, 6,

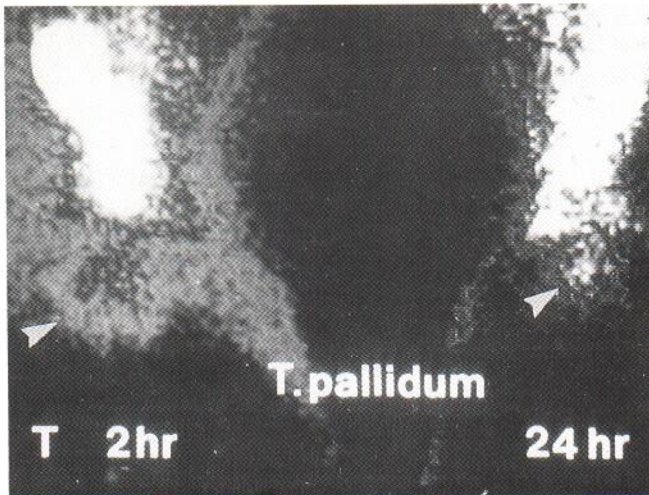

Fig. 1. Scintigraphic images taken at 2 and 24 h after injection of the radiolabelled monoclonal $F(ab')_2$ fragment in a *T. pallidum*-infected rabbit. Testes (*arrowheads*) show increased uptake after 24 h.

8, 12, and 16 h were given to determine the optimum condition. Digested products were purified by serial passage into the protein A-Sepharose CL-4B column and a Sephacryl-200 column (Pharmacia Fine Chemicals AB, Uppsala, Sweden) (9, 10).

Radioisotope labelling

$F(ab')_2$ fragments were labelled with ^{131}I (Korea Atomic Energy Research Institute, Taejon, Korea) with chloramine-T. Approximately 60–70% of the ^{131}I was labelled to yield a specific radioactivity of 1.2–3.5 mCi/mg of the antibody containing less than 3% of free iodide. Rabbit anti-human $F(ab')_2$ specific for γ-chains (Dako Immunochemicals Inc., Copenhagen, Denmark) was labelled by the same method and used as PAb for control against YS-307.

Scintigraphy

Rabbits infected with *T. pallidum* or *S. aureus* were intravenously injected with 1 mCi of the radiolabelled monoclonal or polyclonal $F(ab')_2$ fragments. Images were obtained by a standard field-of-view gamma camera (Siemens Gammasonics, Illinois, U.S.A.) at 2, 24 and 48 h after the injection. A high-energy parallel hole collimator with the energy level of 364 KeV was used. The images of each rabbit were

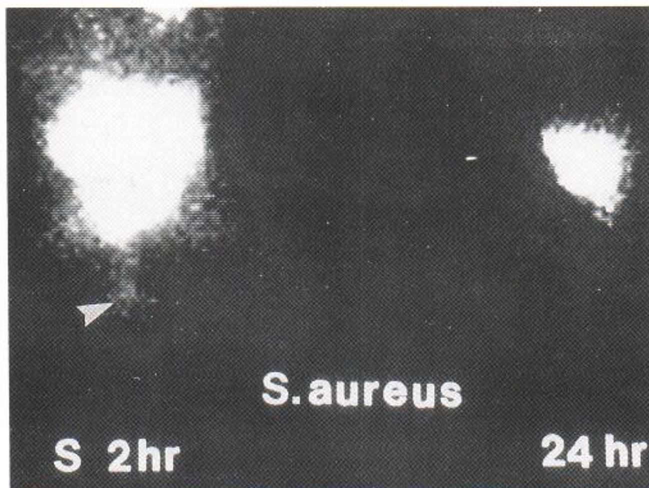

Fig. 2. Scintigraphic images taken at 2 and 24 h after injection of the radiolabelled monoclonal $F(ab')_2$ fragment in a *S. aureus*-infected rabbit. Testes that were visible at 2 h post-injection (*arrowhead*) are not visible after 24 h.

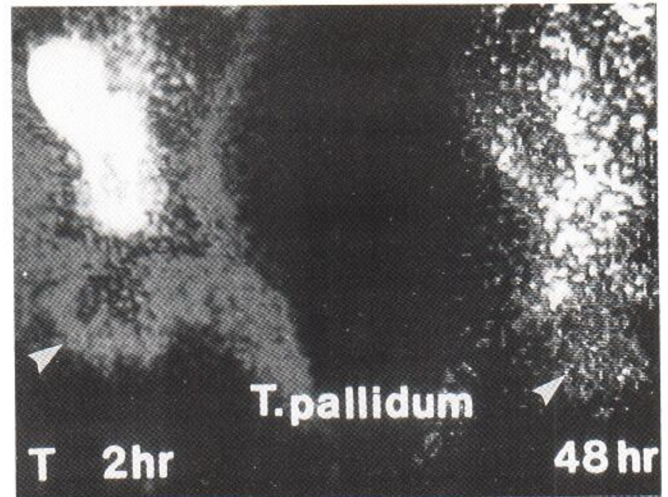

Fig. 3. Under conditions identical with those in Fig. 1, testes (*arrowheads*) are still clearly visible after 48 h.

obtained for a preset time of 300 s. The ratio of radioactivity of the lesion and the thigh area was calculated to obtain a target/background (T/B) radioactivity ratio. Radioactivities of the target (testes) and background (thigh muscles) were separately measured by sacrificing the rabbits 48 h after the inoculation.

Statistical analysis

Data obtained by scintigraphy were analyzed by the Mann-Whitney or Kruskal-Wallis tests, utilizing a SPSS/PC+ program installed in an IBM-compatible personal computer.

RESULTS

When MAb was used, testes infected with *T. pallidum* and *S. aureus* were clearly discernible from the surrounding tissue at 2 h post-injection. At 24 h, *T. pallidum*-infected testes were more prominent (Fig. 1), but the testes of the *S. aureus*-infected gropus were not readily discernible from the surrounding tissue (Fig. 2). *T. pallidum*-infected testes still showed increased uptake at 48 h (Fig. 3). Both groups showed increased uptake at 2 h when polyclonal $F(ab')_2$ was used, but

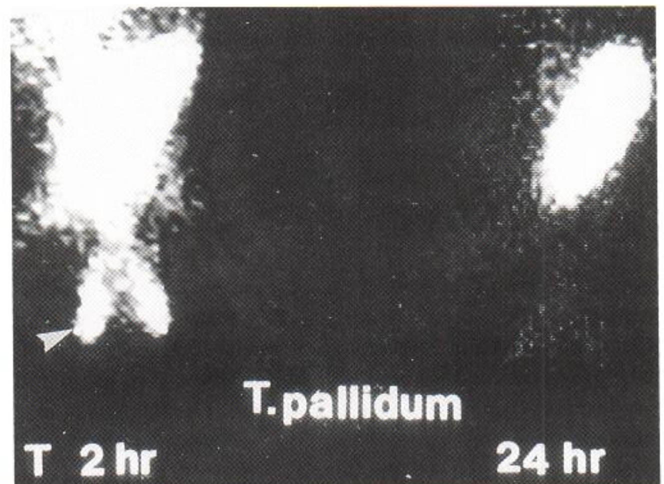

Fig. 4. Images of a *T. pallidum*-infected rabbit injected with radiolabelled polyclonal $F(ab')_2$. Testes seen at 2 h post-injection (*arrowhead*) show no uptake after 24 h.

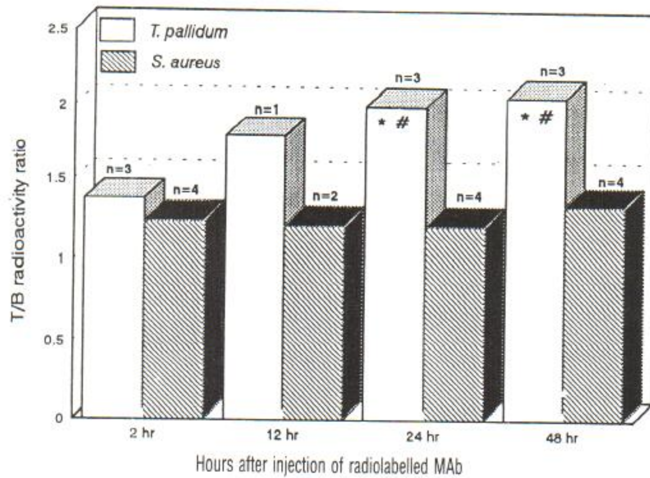

Fig. 5. Sequential variation in testis/background radioactivity ratio when monoclonal antibody to *T. pallidum* was used. Ratios at 24 and 48 h post-injection in the *T. pallidum*-infected groups were significantly higher than the ratio at 2 h post-injection. They were also higher than those of the *S. aureus*-infected groups at the same time interval. *$p<0.05$ by Kruskal-Wallis test when compared with the ratio at 2 h. #$p<0.05$ by Mann-Whitney test when compared with the ratio of the *S. aureus*-infected group at the same time interval.

the images of the testes were not discernible from the surrounding tissue even at 24 h in both groups (Fig. 4).

T/B ratios in the YS-307 injected *T. pallidum*- and *S. aureus*-infected groups were 1.37±0.12 and 1.23±0.08 at 2 h, 2.00±0.36 and 1.20±0.16 at 24 h, and 2.06±0.33 and 1.33±0.24 at 48 h, respectively. The ratios were significantly increased in the *T. pallidum*-infected groups at 24 and 48 h when compared with those of 2 h ($p<0.05$). The ratios of the *T. pallidum*-infected groups at 24 h and 48 h were significantly higher than those of the *S. aureus*-infected groups at the same time interval ($p<0.05$) (Fig. 5). When polyclonal $F(ab')_2$ was used, no sequential or inter-group variations were noted.

After 48 h, the rabbits of the YS-307 injected groups were sacrificed and their testes scintigraphed. The *T. pallidum*-infected group still showed relatively strong uptake, whereas no radioactivity was detectable in the *S. aureus*-infected group (Fig. 6). After the sacrifice, radioactivities of the testes and thigh muscles were estimated by a Well-counter and the results showed that the T/B ratio in the *T. pallidum*-infected group was 3.2±0.52 ($n=3$), which is significantly higher than 1.20±0.12 ($n=4$) of the *S. aureus*-infected group ($p<0.05$).

DISCUSSION

Since the advent of antibiotics, the prevalence of syphilis throughout the world has decreased. VDRL-positive rate in Korea has also decreased from 2.5% in 1977 to 0.4% in 1990 (11). However, partly due to the widespread use of drugs and prostitution, syphilis prevalence in the United States has markedly increased since 1986 (12, 13). Owing to the recent world-wide increase in the number of drug users and the spread of HIV, syphilis may once again become a global health problem.

To effectively handle the disease, more effective means of diagnosing the disease as well as investigating the pathogenesis are needed. Serologic tests are the current mainstay for the diagnosis of phyhilis, but internal organ involvement cannot be detected by the serologic methods alone – nor is it feasible to demonstrate the organism (14–16). Furthermore, in HIV-infected patients, serologic diagnosis may not be possible due to abnormal humoral immune response (17).

Immunoscintigraphy was first introduced to diagnose osteogenic sarcoma (1), using intact antibody to tumour cells. Only recently has it been used for the external detection of infectious diseases (6, 7). In contrast to the intact antibodies used in the previous studies, $F(ab')_2$ or Fab fragments are more readily accumulated in the target tumours and excreted more rapidly from the blood and thus the background activity can be reduced. Non-specific accumulation in the liver can also be reduced (18–20). We have prepared $F(ab')_2$ fragments from MAb specific for *T. pallidum* by pepsin digestion to eliminate the possibility of non-specific binding of the intact immunoglobulin to the Fc receptors on the neutrophils or macrophages in the infection foci. Pepsin digestion was also intended to reduce the background activity.

Rubin et al. (7) applied immunoscintigraphy for the external detection of *Pseudomonas aeruginosa* infection. When radiolabelled *P. aeruginosa*-specific MAb was injected, the images were clearly discernible from the group injected with radiolabelled non-specific antibody from 72 h after injection. In our experiments, the *T. pallidum*-infected group given YS-307 showed adequate contrast with the surrounding tissue from 2 to 48 h after injection. The group infected with *S. aureus* and given YS-307 and the groups infected with *T. pallidum* and *S. aureus* and given PAb showed adequate contrast with the surrounding tissue at 2 h, but the contrast was gone at 24 h. Therefore, the results between specific and non-specific antibodies were readily differentiated from 24 h after the injection. The reason for this discrepancy is probably due to the use of intact immunoglobulin by Rubin et al. (7). The excretion of intact immunoglobulin is protracted due to its longer half-life as compared to $F(ab')_2$ or Fab fragments (21).

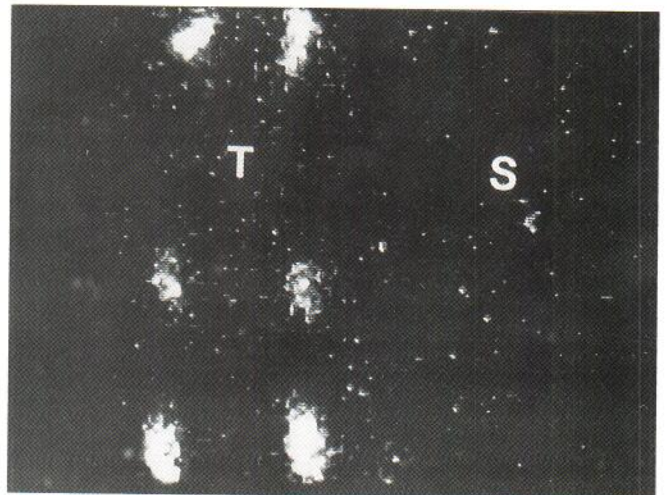

Fig. 6. Extracted testes 48 h post-injection. No radioactivity is seen in the *S. aureus*-infected group in contrast to the *T. pallidum*-infected group. *T*: *T. pallidum*-infected testes. *S*: *S. aureus*-infected testes.

We consider that $F(ab')_2$ fragments of the YS-307, the MAb against *T. pallidum*, can be utilized for immunoscintigraphy to detect internal syphilitic involvement, although further evaluation in human syphilis patients matched with actual demonstration of *T. pallidum* is required for diagnostic as well as investigative purposes.

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