Susceptibility of Murine Transfer Factor to Dimerized Ribonuclease A

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Dialyzable transfer factor was prepared from the spleens of CF1 mice actively sensitized with killed Coccidioides immitis antigen. The transfer factor was administered to normal mice either intraperitoneally or into the hind footpads. The recipient mice were tested for reactivity to the coccidioides antigen and to Candida albicans antigen by means of the footpad swelling test. The transfer factor conferred antigen-specific reactivity upon normal recipient mice when given by the intraperitoneal and footpad routes. This capacity of the transfer factor was destroyed by in vitro pretreatment with dimerized ribonuclease A, an enzyme active against double-stranded, as well as single-stranded, ribonucleic acid. In contrast, monomeric ribonuclease A, which is active against only single-stranded ribonucleic acid under the conditions used here, was without effect upon the transfer factor. These data provide evidence that murine transfer factor contains ribonucleotides that are essential for immunological activity. In addition, the data are consistent with the hypothesis, advanced by others, that the ribonucleotides may be in double-stranded or uniquely looped configurations.

The immunological activity of human transfer factor is resistant to deoxyribonuclease, ribonuclease (RNase), and trypsin but is destroyed by Pronase and snake venom phosphodiesterase (5–7, 9). This enzyme susceptibility pattern also obtains in the murine transfer factor system (8), as reported from this laboratory. Chemical analysis of partially purified human transfer factor has revealed ribose, hexose, ribonucleic acid (RNA) bases, peptide, and lipid phosphorus but not deoxyribose or thymine (1, 2, 10).

Some insight into the structure and mechanism of action of transfer factor might derive from a clearer understanding of its nucleic acid components. Reported here are some investigations of this question in the murine transfer factor system using a dimerized RNase preparation active against double-stranded RNA (3).

MATERIALS AND METHODS

The general procedures have been described previously (8). Briefly, 10- to 12-week-old female CF1 mice were used (Carworth, Inc., Wilmington, Mass.). As the source of transfer factor, mice were actively sensitized with four weekly 0.025-ml intradermal injections of thiomersal-inactivated 0.5% Coccidioides immitis mycelial-phase antigen in saline. One week after the final injection, spleens were collected and transfer factor was extracted according to the procedure of Lawrence (6), as previously reported (8). Spleen cells were harvested and washed in medium 199 (Flow Laboratories, Silver Spring, Md.) and resuspended to a concentration of 10⁶/ml in distilled water. Magnesium sulfate, 50 mg/ml, and a few crystals of deoxyribonuclease (Worthington Biochemicals Corp., Freehold, N.J.) were added, and the mixture was subjected to 10 cycles of freezing-thawing. The resulting lysate was dialyzed twice against 25 volumes of distilled water at 4°C, and the pooled dialysates were concentrated by freeze-drying. The residue was dissolved in sufficient saline so that each milliliter of transfer factor represented approximately three spleens (2 × 10⁶ cells/spleen).

Normal recipient mice were passively sensitized with transfer factor appropriately diluted and administered in standard volumes of 0.25 ml intraperitoneally or 0.025 ml directly into each hind footpad. One day later, each mouse was footpad tested with 0.025 ml of 0.1% C. immitis suspension injected into the right hind footpad and an equal volume of 0.25% heat-killed Candida albicans suspension injected into the left. The thickness of the foot was measured with a dial-type skin thickness gauge prior to antigen injection and again 48 h later. The swelling was recorded in 0.01-mm units.

Some samples of transfer factor were treated with RNase or dimerized RNase prior to administration. Bovine pancreatic RNase A was obtained as protease-free RNase type 1A (90 U/mg; Sigma Chemical Co., St. Louis, Mo.). Dimerized bovine pancreatic RNase A was obtained as a lyophilized preparation (courtesy of Stanford Moore, The Rockefeller University, New York, N.Y.). Enzyme treatment was
carried out in a 0.1 M NaCl-0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.5, overnight at 37°C. Under these conditions, both enzymes were shown to render >93% of a radioactive chick cell RNA preparation acid soluble.

The data were evaluated by analysis of variance.

RESULTS

The samples of transfer factor were prepared in NaCl-Tris buffer. The first contained only 1:5 coccidioides-specific transfer factor. The second contained transfer factor and dimerized RNase A, 10 μg/ml. The two preparations were incubated overnight at 37°C. Each preparation was then injected into two groups of six normal mice each. One group was given 0.25 ml intraperitoneally, and the second group was given 0.025 ml into each hind footpad. On the following day, all mice were given a coccidioides antigen footpad test in the right hind footpad and candida antigen in the left. An additional group of six normal mice was given footpad antigen tests only. Forty-eight hours later, the footpad swelling was measured (experiment A, Table 1).

Untreated transfer factor induced antigen-specific reactivity when given intraperitoneally and into the footpads. Dimerized RNase A pretreatment of the transfer factor caused a slight, but not statistically significant, decrease in the reactivity after intraperitoneal injection (49.2 versus 39.3). This slight diminution of transfer factor potency by dimerized RNase A, however, was not apparent in the footpad-inoculated groups (41.5 versus 39.5).

Because of this suggestion of some inactivation of transfer factor by dimerized RNase A, the above experiment was modified and repeated. In this experiment (B), three samples of transfer factor were prepared; the first contained only 1:5 transfer factor in NaCl-Tris buffer, the second contained 1:5 transfer factor plus RNase A, 40 μg/ml, and the third contained 1:5 transfer factor plus dimerized RNase A, 20 μg/ml. The three samples were incubated at 37°C, diluted in equal volumes of NaCl-Tris buffer to provide transfer factor in a dilution of 1:10, and tested by intraperitoneal and footpad inoculation in groups of 10 mice each (experiment B, Table 1). Under the conditions of this assay, there was inactivation by dimerized RNase A of the transfer factor given intraperitoneally (32.2 versus 6.8) and into the hind footpads (31.6 versus 2.6). In contrast, RNase A was without effect.

It is apparent that the dimerized RNase acted upon the transfer factor itself rather than upon the recipient mouse in preventing the antigen-specific footpad reactivity (experiment B), because in experiment A the same amount of dimerized RNase A was given as in experiment B without preventing reactivity (Table 1).

DISCUSSION

Human and murine transfer factors are sensitive to phosphodiesterase but resistant to de-

| Table 1. Susceptibility of coccidioides-specific transfer factor to RNase |
|-----------------------------|----------------|-----------------|-----------------|
| Expt | Transfer factor | Enzyme treatment | No. of mice | Footpad test antigen* |
| Route | Dilution vol | | Coccidioides | Candida |
| A | Intraperitoneal | 1:5 | Untreated | 6 | 49.2 (5.65) | 1.3 (1.30) |
| | | 0.25 ml | Dimerized RNase A | 6 | 39.3 (3.52) | 0.7 (0.95) |
| Footpads | 1:5 | Untreated | 6 | 41.5 (5.81) | 0.3 (0.71) |
| | | 0.025 ml | Dimerized RNase A | 6 | 39.5 (2.99) | 1.3 (1.22) |
| None | | | 6 | 1.3 (0.88) | 0.7 (0.76) |
| B | Intraperitoneal | 1:10 | Untreated | 10 | 32.2 (2.28)* | 0.4 (0.58)* |
| | | 0.25 ml | RNase A | 10 | 31.5 (2.97)* | 0.8 (0.20)* |
| | | | Dimerized RNase A | 10 | 6.8 (2.28)* | -0.1 (0.62)* |
| Footpads | 1:10 | Untreated | 10 | 31.6 (3.20)* | 0.7 (0.42)* |
| | | 0.025 ml | RNase A | 10 | 28.8 (3.28)* | 0.2 (0.39)* |
| | | | Dimerized RNase A | 10 | 2.6 (1.28)* | -0.5 (0.34)* |
| None | | | 10 | 1.8 (0.44)* | 0.6 (0.58)* |

* Mean footpad swelling in 0.01-mm units (standard error) at 48 h after antigen injection.  
* Right hind footpad.  
* Left hind footpad.  
* Into each hind footpad.  
* For experiment B, a > b; P < 0.01.
oxyribonuclease and RNase (5-9). The experiments reported here were designed to test the nature of the phosphodiesterase-labile component of murine transfer factor. The test system, as reported previously (8), used transfer factor extracted from spleens of mice immunized with killed C. immitis vaccine. Reactivity in the recipients of transfer factor was assayed by the footpad swelling method.

The data presented here confirm the prior report of antigen-specific transfer activity and bovine pancreatic RNase A resistance of murine transfer factor (8). In the present study, transfer factor was also tested for susceptibility to dimerized bovine pancreatic RNase A. This enzyme, which is prepared by dimethyl suberimidate cross-linking of RNase A, is active against double- as well as single-stranded RNA (3). The coccidioides-specific murine transfer factor was found to be inactivated by the dimerized RNase, and this result was obtained with transfer factor administered either intraperitoneally or directly into the footpads.

The destruction of transfer factor activity by dimerized RNase A provides the first direct evidence that ribonucleotides are essential components of this immunological material. The susceptibility of the transfer factor to dimerized RNase A, with concomitant resistance to its monomeric form, could represent evidence that the ribonucleotides are in a double-stranded configuration, as suggested by Lawrence (unpublished observations). However, it should be noted that Crawford et al. (4) could not detect double-strandedness in a human transfer factor preparation using the ethidium bromide test. Dunnick and Bach (5) point out that transfer factor might be resistant to RNase simply because the RNA residues are small, unusual in composition, or marked by other components. Whatever the reason for the susceptibility of murine transfer factor to dimerized RNase A, this feature provides a new enzyme marker for transfer factor activity. To determine whether human transfer factor also is susceptible to dimerized RNase A will require direct testing.

Human transfer factor is reported to be sensitive to Pronase (5, 9), and we have found this enzyme susceptibility also in transfer factor from mice (8). Thus, murine transfer factor appears to contain ribonucleotides and peptides, both of which are essential for its immunological activity. The mechanism by which antigen-specific reactivity is encoded and transferred by this ribonucleotide-peptide molecule is of interest and potential importance, and the model murine system reported from this laboratory provides a tool for further exploration of these questions.

LITERATURE CITED


