AFFINITY CONSTANTS OF ANTI-LEPTOSPIRA MONOCLONAL ANTIBODIES FOR LEPTOSPIRAS, NUMBERS OF ANTIGENIC DETERMINANTS ON LEPTOSPIRAS, AND THEIR INFLUENCE ON ELISA AND THE MICROSCOPIC AGGLUTINATION TEST

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The affinity constants of monoclonal antibodies and the numbers of antigenic determinants on the serovars of *Leptospira interrogans* were determined by Scatchard analysis, and their effects on the reactivity patterns, examined by ELISA and the microscopic agglutination test (MAT), were discussed. Anti-*canicola* monoclonal antibody CT3 and 4 anti-*hebdomadis* monoclonal antibodies were examined with the serovars of Canicola and Hebdomadis serogroups, respectively.

The results examined by ELISA and MAT agreed in many combinations of monoclonal antibodies and serovars. However, there was a considerable number of combinations showing a discrepancy, such as positive in MAT but negative in ELISA. There was only one combination (CT3 and *galtoni*) showing positive in ELISA but negative in MAT.

The affinity constants were different among various combinations of monoclonal antibodies and serovars. The highest affinity constant of anti-*canicola* CT3 was 4.20×10^8/M for *galtoni* and the lowest affinity constant of the same monoclonal antibody was 4×10^6/M for *sumneri*. Affinity constants of anti-*hebdomadis* monoclonal antibodies ranged from 1.89×10^8/M (Hw4 and *kremastos*) to 6×10^6/M (Hw1 and *maru*). The number of antigenic determinants per leptospira organism recognized by the monoclonal antibodies was at the level of 10^5 to 10^6, except for that recognized by Hw5, which was at the level of 10^4.

From the results obtained, the affinity constant seemed to play an important role in the serological tests; the combinations of monoclonal antibodies and serovars showing affinity constants of more than 10^7/M reacted both in ELISA and MAT, while those showing affinity constants at the level of 10^6/M reacted in MAT but not in ELISA. The fact that the combinations whose affinity constant was at the level of 10^6/M were detected only by MAT may explain the discrepancy in which MAT was positive but ELISA was negative.

There is presently a movement to use monoclonal antibodies instead of immune sera in the clssification of leptospira. However, the results of ELISA and MAT using monoclonal antibodies do not always agree, as shown in the present study. Therefore, the affinity constant should be considered in the classification of leptospira using monoclonal antibodies.