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Multiple organ-reactivity of monoclonal autoantibodies to mouse erythrocytes

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Summary. Autoantibodies reacting with bromelain-treated autologous mouse red blood cells (Br-MRBC) are spontaneously produced by normal mice. In order to understand the biological significance of these autoantibodies, anti-Br-MRBC monoclonal autoantibodies have been prepared and studied for reactivity with a panel of frozen tissue sections from organs of normal mice by direct immunofluorescence. It has been found that the anti-Br-MRBC monoclonal autoantibodies are polyspecific, since they react with cells in multiple organs.
Key words. Br-MRBC; monoclonal antibodies; autoimmunity.

Lymphoid tissues of normal mice contain cells that secrete autoantibodies to autologous mouse red blood cells (MRBC) treated with bromelain (Br), a proteolytic enzyme[1,2]. The biological significance of anti-Br-MRBC autoantibodies is not known, but this phenomenon seems to constitute a model of spontaneous autoimmunity for at least two reasons: first, the bromelain treatment does not create a new antigen, but only reveals a 'hidden' autoantigen[3]; second, the development of anti-Br-MRBC antibodies is inhibited by suppressor T lymphocytes and by the presence of the autoantigen[3-6], as in the case of other models of autoimmunity.

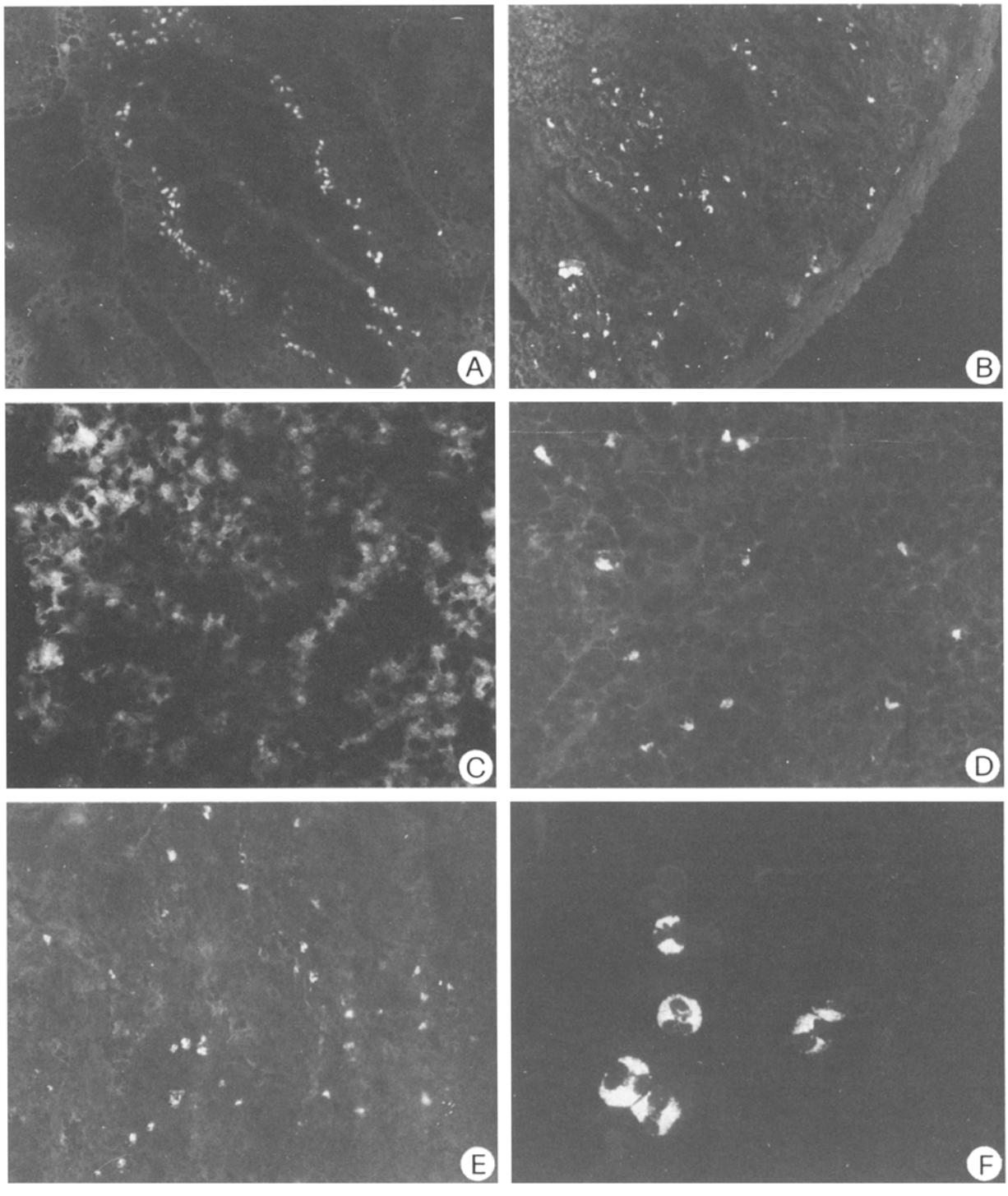
Either a cross-reacting environmental antigen or a self-antigen present in the body are thought to activate the immune response to autologous Br-MRBC. Several arguments, however, favor the latter possibility; for example, germ-free and pathogen-free mice have the same number of cells making antibodies to Br-MRBC as conventionally reared mice of the same strain[3]; moreover, the antigen binding to Br-MRBC antibodies has been isolated both from erythrocytes and serum[7]; finally, anti Br-MRBC antibodies also bind to the Fc portion of the IgG molecule[8].

To know whether normal mice express other autoantigens capable of binding to Br-MRBC antibodies, we have prepared anti-Br-MRBC monoclonal autoantibodies, using peritoneal cells as fusion partners[5], and we have studied their reactivity with a panel of tissue sections from different organs of normal mice.

Materials and methods. Peritoneal cells (PC) from 2–3-month-old inbred Balb/c female mice were collected by injecting into the peritoneal cavity 5 ml of RPMI-1640 (Flow Laboratories, Rickmansworth, U.K.) supplemented with 1% fetal bovine serum (FBS), and 5 U/ml heparin. The cells were pooled, washed three times at 400 g for 8 min at 4 °C, and then 15×10^6 PC were fused with 7.5×10^6 P3.X63.AG8.653 nonsecretor myeloma cells by using 1 ml of 50% polyethylene glycol (MW 1000) at neutral pH. Fused cells were washed and suspended in growth medium (RPMI-1640 with 10% FBS, 2mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.2 µg/ml fungizone) containing hypoxanthine (100 µM), aminopterin (0.4 µM), and thymidine (16 µM) (HAT medium), and seeded in 0.2 ml volume at 2×10^5 cells/well in 96-well plates. Hybrids were fed by replacing 100 µl of HAT medium twice a week for the first two weeks, and then cultured for five more days in HT medium.

Outgrowing hybrid cells were tested for the production of autoantibodies to Br-MRBC by a plate modification of the Jerne and Nordin plaque-forming cell (PFC) assay[9], using, as target, mouse red blood cells (MRBC) obtained by bleeding Balb/c mice from the axillary vein under ether anesthesia, and immediately treated with bromelain (Serva Feinbiochemica, Heidelberg, FRG), as previously reported[6]. The cultures producing autoantibodies to Br-MRBC were cloned twice by limiting dilution on feeder cells consisting of 5×10^3 peritoneal cells per well collected from normal Balb/c mice. The hybrid cell clones producing autoantibodies to Br-MRBC were expanded and the culture supernatants were precipitated with ammonium sulphate (50% of saturation) and passed through anion-exchange cromatography column. The fractions showing hemolytic activity in the presence of complement were pooled and labeled with fluorescein isothiocyanate (FITC; Sigma Co., St. Louis, Mo., USA) by dialyzing the antibody solution against a 10-volume solution of FITC (100 µg/ml) in 0.05 M bicarbonate buffered saline, pH 9.2, at 4 °C overnight. The reaction was stopped by changing the dialysis buffer to 0.02 M phosphate-buffered saline pH 7.0 (PBS). The free FITC was removed from the conjugated by chromatographing labeled samples on Sephadex G-25 in PBS. The fluorescein-conjugated monoclonal antibodies were then tested for reactivity with a panel of frozen tissue sections of normal organs from Balb/c mice by incubating each section with a 1:20 dilution of fluorescent antibody in PBS for 1 h at room temperature and then by washing three times in PBS. Tissue sections were from thyroid, salivary glands, adrenals, ovary, testis, epididymis, heart, lung, pancreas, kidney, liver, stomach, intestine, pituitary, thymus, and lymph node; the monoclonals were also tested on smears of acetone-fixed bone marrow cells. A fluorescein-labeled IgM monoclonal antibody to Coxsackie B3 virus (Garzelli et al., manuscript in preparation) was used as negative control.

Results. Peritoneal cells from normal Balb/c mice were fused by polyethylene glycol with mouse myeloma cells and the fusion products were distributed into a microtiter plate. Of the 96 wells that were seeded, 44 showed cell growth. These cultures were tested for autoantibodies to Br-MRBC by hemolytic PFC assay. Two hybrid cell cultures were found to lyse bromelain-treated mouse erythrocytes in the presence of complement; they were, however, unable to lyse normal erythrocytes. These two autoantibody-producing hybrids were cloned twice by limiting dilution and two stable sub-



Pattern of reactivity of monoclonal antibody F12-3-3 with frozen sections of *A* testis, *B* stomach, *C* salivary gland, *D* thymus, *E* inguinal lymph node, and *F* with a smear of acetone-fixed bone marrow cells, as detected by direct immunofluorescence.

clones, coded G3-2-3 and F12-3-3, growing independently from the two parent cell cultures and producing monoclonal antibody of the IgM class were obtained.

The culture supernatants of these hybrids were precipitated with ammonium sulphate, passed through an ion-exchange chromatography column, labeled with fluorescein, and then tested by direct immunofluorescence for reactivity with a panel of frozen sections from a variety of organs of normal mice. The two monoclonals were found to react identically with cells, other than erythrocytes, in different organs. Figure 1 shows the pattern of tissue reactivity of F12-3-3 monoclonal autoantibody. In particular, it reacted with cells close to the lumen of seminiferous tubules of testis (fig. A), with cells in the lamina propria of gastric mucosa (fig. B), with cytoplasm of cells in the salivary glands (fig. C). It also reacted with cells scattered in lymphoid organs, including

thymus (fig. D) and lymph node (fig. E), and with the cytoplasm of approximately 20% of nucleated cells of bone marrow (fig. F).

Discussion. The capacity of anti-Br-MRBC autoantibodies to react with antigens in multiple organs may be seen in the context of polyreactivity of monoclonal IgM autoantibodies. Both in mice and humans, in fact, most monoclonal IgM autoantibodies described so far, isolated from normal individuals or from patients and animals with autoimmune disorders, when studied in detail, turned out to be polyreactive[10,11] and often to react with antigens in a number of different organs[12–16]. The biochemical basis of multiple organ-reactivity of monoclonal autoantibodies seems to be the presence of common antigens or epitopes in different organs, or similar, but not identical, epitopes to which the autoantibody can structurally accommodate[17]. Although the significance of multiple organ-reactive IgM autoantibodies is still undefined, the constancy of finding these autoantibodies suggests that they are a common feature of the normal B cell repertoire. A possible answer to the question of the role of these autoantibodies might come from the recent discovery of the 'Ly-1 B' cells. In normal and autoimmune NZB mice, in fact, these cells constitute a functionally distinct B cell subpopulation, bearing surface IgM and IgD and Ly-1 B antigens[18,19]. The Ly-1 B cell subpopulation is thought to play some important role in autoimmunity because it contains virtually all the cells that spontaneously secrete IgM autoantibodies to several commonly studied autoantigens, such as Br-MRBC, thymocytes and single-stranded DNA, but only minimal numbers of cells that give an IgM response to exogenous antigens[19]. One possibility could be that Ly-1 B cells produce autoantibodies to aged or dead cells or tissues expressing 'hidden' autoantigens, in order to favor their clearance from the body. If this is the case, polyreactivity of these autoantibodies would endow the immune system with an economy for antigen recognition.

Multiple organ-reactivity, however, is not only restriced to IgM autoantibodies. It has been, in fact, recently reported that anti-idiotypic antibody to a multiple organ-reactive monoclonal IgM autoantibody can induce IgG antibodies showing the same pattern of tissue reactivity as the IgM monoclonal autoantibody[20]. This raises the possibility of a further mechanism to explain the occurrence of an IgG-mediated autoimmune response to certain tissues or organs; for example, if a tissue is injured, B lymphocytes committed to producing autoantibodies to epitopes in multiple organs, upon exposure to any of these epitopes, could be triggered to the production of IgM autoantibodies which are necessary for the clearance of the injured tissue; this response will be followed, according to Jerne's network theory, by an anti-idiotype response that, in turn, will trigger an anti-anti-idiotype response. This could be directed not necessarily only to the tissue that was initially injured and that initiated the cascade, but also to any tissue expressing that epitope. Thus, the existence of 'normal' multiple organ-reactive autoantibodies could turn to be one of the key mechanisms for triggering autoimmune responses.

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Changes in intracellular pH and cell volume during the early phase of DMSO-induced differentiation of Friend erythroleukemia cells[1]

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Summary. Changes in intracellular pH and water volume were measured after treatment of Friend erythroleukemia cells with 1.5% DMSO. It was found that a continuous decrease in pH_i occurred, beginning 1 h after induction and a decline in pH_i of 0.18 was measured after 9 h. In addition a decline in cellular water volume, of 12% only 15 min after induction, and 23% after 9 h, was observed.

Key words. Friend cells; differentiation; intracellular pH; cell volume.

The intracellular pH appears to play an important role in the regulation of cell growth. An elevated pH_i corresponds with mitotic activity in various mammalian cell systems[4,5]. Treatment with mitogens leads to a quick alkalinization of the