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Enhanced Splenic ATPase Activity in Immunized Mice[1] (38364)

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Despite marked advances in the understanding of mechanisms involved in immune responses there is still very little knowledge about enzymes mediating these events. In contrast to extensive studies concerning enzyme activities in many other biological processes only a few groups of enzymes have been examined in regards to their role in immunologic events, *i.e.*, enzymes involved in nucleic acid metabolism (1–4) and lysosomal enzymes involved in antigen degradation (5, 6). However, increasing attention has recently been directed to adenyl cyclase, which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) and its role in cellular metabolism, including lymphoid cells. It is now accepted that this cyclic nucleotide also stimulates biochemical processes associated with antibody formation (7, 8). In the present study the activity of ATPase, which also utilizes ATP as a substrate, was examined in spleen cells of immunized mice as a function of the immune response.

Methods and Materials. Animals. Inbred Balb/c mice were obtained from Cumberland View Farms, Clinton, TN and were 6–8 weeks of age at the time of testing. They were housed in groups of six to ten in plastic cages and fed water and Purina mouse pellets *ad libitum*.

Antigen. The mice were immunized by an intravenous (iv) injection with 0.5 ml of a 2% suspension of washed sheep red blood cells (4×10^8 SRBC), except where indicated.

Assessment of immunity. The hemolytic plaque assay was used for enumerating individual plaque forming cells (PFC) against sheep erythrocytes using the plaque assay developed by Jerne and Nordin (9). In brief, the number of PFCs per spleen or per million spleen cells tested was determined by incubating an aliquot of a washed spleen cell suspension from individual mice in agar gel in Petri plates. The number of direct (IgM) PFCs was determined for individual spleens at various times after immunization.

ATPase determination. ATPase activity of individual spleens of normal and immunized mice was assayed using cell homogenates (10). The spleens were dispersed in Hanks' solution through a nylon filter into a single cell suspension. The cells were washed twice with ice cold 0.15 *M* NaCl, once with 0.15 *M* LiCl buffered with 0.01 *M* Tris–HCl, pH 7.4 and homogenized in 0.25 *M* sucrose in 0.1 *M* Tris–HCl buffer, pH 7.4, using a motor driven (TRI-R) glass homogenizer with a Teflon tipped pestle at maximum speed for 2 min. All subsequent steps were carried out at 0–4° . The homogenates were immediately assayed for enzyme activity. The reaction mixture for the ATPase assay contained, in a final volume of 1.2 ml, 0.25 *M* sucrose, 30 m*M* Tris–HCl buffer, pH 7.6, 6 m*M* $MgCl_2$, 3 m*M* Tris–ATP, and, when added, 100 m*M* NaCl and 15 m*M* KCl. After 30 min incubation at 37° the tubes were cooled in an ice bath for 2 min and ice cold trichloroacetic acid was added to a final concentration of 5%. The precipitate was removed by centrifugation and the concentration of free inorganic phosphate present in the system was determined. Total ATPase activity was calculated from the activity in the presence of $MgCl_2$, NaCl and KCl. Inorganic phosphate was determined by the method of Fiske and Subbarow (11). For each experiment the ATPase level and PFC number were assayed for spleens of 5–6 mice per group.

Results. Immunization of mice with SRBCs

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resulted in a significant increase in total ATPase activity in the spleens of the experimental animals (Table I). Within 2–4 days after immunization there was a 85–125% increase in activity per spleen. By day 8 the level of ATPase activity returned to normal levels. Calculation of ATPase activity per mg spleen also showed a rise in activity, peaking at day 4, but the percent increase was less than that observed when calculated per whole spleen. This appeared due to the marked increase in spleen weight over a 4–6 day period after immunization. However, the increase in ATPase activity per milligram spleen was at a much greater level than the increase in spleen weight.

As is evident in Fig. 1, the rise in enzyme activity per spleen coincided in time with appearance of antibody PFCs, which is considered a precise indicator of immunity at the cell level following immunization with sheep RBCs. The peak day of the antibody response, at the level of individual PFCs, occurred on days 4–5, the time of the peak enzyme activity. During the second week following immunization, when the number of PFCs gradually decline to near background levels, ATPase activity also returned to normal levels or even lower.

In order to relate immune reactivity to ATPase activity, experiments were performed with different antigen concentrations. A relationship was observed between antigen dose and the peak response of both antibody forming cells and ATPase activity (Table II). A dose of 4×10^8 SRBCs, previously shown to be maximally effective in stimulating the highest PFC responses in mouse spleens (14, 15), resulted in the largest antibody plaque responses and also stimulated the highest ATPase activity. Increasing the antigen dose 10-fold resulted in a reduced PFC response and a similarly smaller effect on ATPase activity.

TABLE I. Effect of Immunization of Mice with Sheep RBCs on Changes in Splenic ATPase Activity and Spleen Weights.

Time in days after immunization[a]	Average spleen weights (mg)	ATPase activity[b]	
		Total ATPase	Mg^{2+}–ATPase
0	98 ± 18	100	100
+1	102 ± 22	118 ± 5	116 ± 3
+2	119 ± 16	148 ± 9	132 ± 6
+3	138 ± 32	207 ± 6	188 ± 5
+4	131 ± 22	227 ± 12	195 ± 15
+8	122 ± 18	122 ± 10	122 ± 6

[a] Groups of mice injected iv with 4×10^8 sheep RBCs and assayed for ATPase activity on day indicated.

[b] ATPase activity per spleen (± SD) for four to six mice on day indicated expressed as percent of control activity for nonimmunized mice.

Most of the ATPase activity increase appeared due to the Mg^{2+}-ATPase form, since addition of NaCl and KCl to the reaction mixture, a necessary ingredient to activate the membrane bound Na+K+-ATPase, did not result in much additional activity. Thus no significant increase in Na^+K^+-ATPase activity could be related to immune reactivity. Indeed, in some experiments a slight overall decrease in Na^+K^+-ATPase activity was noted. Furthermore, ouabain, which is a potent inhibitor of Na^+K^+-ATPase (10), did not significantly affect the activity of the splenic ATPase activity, either in normal or immunized mice. Even a concentration of 10^{-4} *M* had no significant inhibiting effect.

Discussion. These results show that stimulation of the immune response, characterized by a marked proliferation of immunocompetent cells, is associated with a corresponding increase in the level of Mg^{2+}-ATPase activity. However it should be noted that, despite the rapid rise in the number of antibody forming cells in the spleens of SRBC stimulated mice, the increased number of such hemolysin secreting cells never reaches a level of more than 0.1% of the total number of splenic lymphoid cells. Athough this increase represents a several thousand-fold rise in the number of antibody forming cells as compared to the number of "background" PFCs in the spleens of nonimmunized mice, the total number of these cells represents only a small fraction of the spleen cell population. Therefore, it seems even more significant that in the whole spleen the increase in enzyme activity was in the range of 100–125% as compared to the several thousand percent increase in the number of PFCs. This suggests that the ATPase activity is either more pronounced in individual antibody forming cells as compared to other splenic cells, or that increased levels of ATPase activity occur also in lymphoid cells not actually involved in secreting antibodies. The latter possibility seems likely, since it is well documented that marked changes in cellularity occurs after immunization

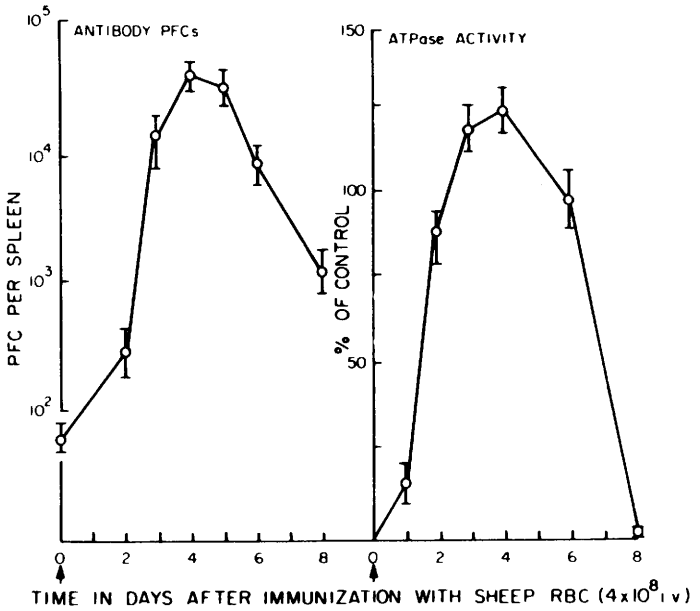

FIG. 1. Comparative increases in the number of antibody plaque forming cells and total ATPase activity in spleens of mice immunized iv with 4×10^8 sheep RBCs. Each point represents average response of four to six mice on the day indicated after immunization. ATPase activity is represented as percent increase of control values for each test day.

and that only a fraction of these cells produce antibodies (14, 15).

It is known that several distinct cell types interact during antibody formation (12, 13). These cells include macrophages which are considered antigen-processing cells, at least for particulate antigens such as sheep erythrocytes, as well as B− and T−lymphocytes and plasma cells which are derived from B−cells and secrete antibodies. That no significant increase in ATPase activity was found in the first 24 hr after immunization and that the greatest increase in enzyme activity coincided with the peak response, suggests that this enzymatic activation is associated with the synthesis or release of antibodies and not with antigen processing. The elevated ATPase activity may reflect either the increased energy requirements of cells associated with antibody formation and/or the energy requirements necessary to accommodate the marked increase in cell proliferation which occurs in the spleen after immunization. Continuing investigations concerning changes in ATPase activity following antigenic stimulation

TABLE II. Effect of Antigen Dose on Splenic ATPase Activity and Antibody Plaque Response in Mouse Spleens.

Antigen dose for immunization[a]	Average spleen weights (mg)	Response per spleen (+4 days)[b]	
		ATPase (percent of controls)	Antibody PFCs
None	97 ± 22	100	98 ± 18
4×10^6	102 ± 18	93 ± 5	4,650 ± 480
4×10^7	118 ± 20	197 ± 11	37,650 ± 6,890
4×10^8	138 ± 32	238 ± 12	58,800 ± 12,600
4×10^9	148 ± 86	120 ± 9	32,500 ± 6,500

[a] Groups of mice injected iv with indicated dose of RBCs 4 days before assay.

[b] Average response of groups of four to six mice injected with indicated dose of antigen. ATPase activity (mg^{2+}) expressed as percent of control activity (± SD) for immunized mice.

should provide additional information concerning biochemical events involved in the immune response.

Summary. The ATPase activity in spleens of mice immunized with sheep erythrocytes was examined. A maximal increase in ATPase activity was found at the time when peak numbers of specific antibody forming cells were evident in the mouse spleen, 4–5 days after immunization. A relationship was observed between antigen dose and the peak response of both antibody PFCs and ATPase activity. The Mg^{2+}-ATPase activity was specifically enhanced, while no increase in $Na^{+}K^{+}$-ATPase activity was demonstrable. These results suggest that a relationship exists between Mg^{2+}-ATPase activity and antibody formation in mouse spleens.

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