deficiencies on cellular protein synthesis in eukaryotes show that deficiency causes a very rapid decrease in protein synthesis, a significant reduction in charged tRNA, diminished ribosome content, a dissolution of polysomes into monosomes and marked impairment of the initiation of protein synthesis (Juergen and Pogo, 1974; Vaughan et al., 1971; Allen et al., 1969; Vaughan and Hansen, 1973). The dependence of RNA synthesis upon simultaneous protein synthesis is a significant regulatory mechanism in both prokaryotes and eukaryotes. This stringent control has been characterized in simple eukaryotes such as yeast and appears to occur primarily at the transcription of rRNA (Schulman et al., 1977). The mechanism of this control involves the binding of RNA polymerases to the nuclear DNA template. On the basis of our present data, it is reasonable to speculate that the mechanism of the amino acid dependence of insulin stimulation of macromolecule synthesis may involve similar mechanisms to those described for other eukaryotes. Further studies are necessary to substantiate this thesis.

References

Allen, R. E., Raines, R. L., and Regen, D. M. (1969), *Biochim. Biophys. Acta 190,* 323.

Baseman, J. B., Paolini, D., and Amos, H. (1974), *J. Cell Biol. 60,* 54.

Drezner, M. K., Eisenbarth, G. S., Neelon, F. N., and Lebovitz, H. E. (1975), *Biochim. Biophys. Acta 381,* 384.

Ebert, P. S., and Prockop, D. J. (1967), *Biochim. Biophys. Acta 136,* 45.

Eisenbarth, G. S., Beuttel, S. C., and Lebovitz, H. E. (1973), *Biochim. Biophys. Acta 331,* 397.

Eisenbarth, G. S., Drezner, M. K., and Lebovitz, H. E. (1975), *J. Pharmacol. Exp. Ther. 192,* 630.

Hall, K., and Uthne, K. (1971), *Acta Med. Scand. 190,* 137.

Hintz, R. L., Clemmons, D. R., Underwood, L. E., and Van Wyk, J. J. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2351.

Jeffay, H., Funso, O. O., and Jewell, W. R. (1960), *Anal. Chem. 32,* 306.

Juergen, K., and Pogo, A. (1974), *J. Biol. Chem. 249,* 568.

Lebovitz, H. E., and Eisenbarth, G. S. (1975), *Vitam. Horm. (N.Y.) 33,* 575.

Peck, W. A., and Messinger, K. (1970), *J. Biol. Chem. 245,* 2722.

Randerath, K., and Randerath, E. (1967), *Methods Enzymol. 12,* 323.

Rillema, J. A. (1975), *Am. J. Physiol. 228,* 1531.

Salmon, W. D. (1960), *J. Lab. Clin. Med. 56,* 673.

Salmon, W. D., DuVall, M. R., and Thompson, E. Y. (1968), *Endocrinology 82,* 493.

Schulman, R. W., Sripati, C. E., and Warner, J. R. (1977), *J. Biol. Chem. 252,* 1344.

Van Wyk, J. J., Underwood, L. E., Baseman, J. B., Hintz, R. L., Clemmons, D. R., and Marshall, R. N. (1975), *Adv. Metab. Disord. 8,* 127.

Vaughan, M. H. and Hansen, B. S. (1973), *J. Biol. Chem. 248,* 7087.

Vaughan, M. H., Pawlowski, P. J., and Forchhammer, J. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 2057.

Accumulation of Immunoglobulin Messenger Ribonucleic Acid in Immunized Mouse Spleen†

Tasuku Honjo,* Akira Shimizu, Masa-aki Tsuda, Shunji Natori, Tohru Kataoka, Chieko Dohmoto, and Yoshitake Mano

ABSTRACT: We have measured the concentration of mRNAs coding for immunoglobulins, κ and λ type light chains and γ_1 type heavy chain, in mouse spleen cells activated by bacterial lipopolysaccharide or sheep red blood cells. These mRNAs were quantitated by hybridization to radioactive DNA complementary to highly purified immunoglobulin mRNAs from mouse myelomas. In the lipopolysaccharide-stimulated spleen cells, only light chain mRNA accumulated, whereas γ_1 type heavy chain mRNA remained unvaried. The light chain mRNA concentration also increased in purified bone-marrow-derived lymphocytes. The lipopolysaccharide-induced light chain mRNA was similar to light chain mRNAs purified from myelomas. The accumulation and disappearance of light chain mRNA in bone-marrow-derived lymphocytes coincide with the kinetics of synthesis of immunoglobulin M which is the major species induced by lipopolysaccharide. In sheep red blood cell stimulated spleen, the specific accumulation of κ type light chain and γ_1 type heavy chain mRNAs parallels immunoglobulin G synthesis. These results seem to indicate that the increment of immunoglobulin mRNA concentration in bone-marrow-derived lymphocytes is important for induction of immunoglobulin synthesis.

In order to elucidate the molecular mechanisms of the immune response, it is essential to know which step(s) of antibody synthesis is stimulated upon activation of immunocompetent lymphocytes. A vital question is whether induction of antibody synthesis is due to activation of Ig[1] gene transcription or of translational steps of preexisting Ig mRNA. Purification of

† From Department of Physiological Chemistry and Nutrition, Faculty of Medicine and Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo, Japan. *Received April 25, 1977; revised manuscript received September 1, 1977.* This investigation was supported by grants from Jane Coffin Child Memorial Fund for Medical Research, from Matsunaga Science Foundation, and from Ministry of Education, Science and Culture of Japan.

[1] Abbreviations used are: Ig, immunoglobulin; L_κ and L_λ chains, κ and λ type light chains; H_{γ_1} chain, γ_1 type heavy chain; LPS, lipopolysaccharide; SRBC, sheep red blood cells; C_rt, the product of the concentration of RNA and the time of incubation; T and B lymphocytes, thymus-derived and bone-marrow-derived lymphocytes; poly(A), poly(adenylic acid).

Ig L and H chain mRNAs and their reverse transcription have provided highly radioactive DNA complementary to the Ig mRNA for a nucleic acid hybridization probe to quantitate mRNA (Honjo et al., 1974, 1976a,b; Faust et al., 1974; Stavnezer et al., 1974; Rabbitts, 1974; Schechter, 1975; Ono et al., 1977a,b). Using this probe, we have measured the Ig mRNA content in mouse spleen cells which had been immunized with bacterial LPS or SRBC.

LPS which is commonly termed B lymphocyte mitogen activates polyclonal B lymphocytes to induce IgM synthesis in the absence of any accessory cells such as T lymphocytes or macrophages (Coutinho and Möller, 1975). In contrast a normal antigen like SRBC requires cooperation of the accessory cells to induce IgM and IgG syntheses in B lymphocytes.

We will report here that Ig mRNA accumulates and disappears specifically coincidental to Ig synthesis in the LPS (or SRBC)-stimulated spleen. Accumulation of Ig mRNA is due to the increased concentration of mRNA in a B lymphocyte but not due to the increased number of B lymphocytes containing a constant amount of Ig mRNA.

Experimental Procedures

(a) *Animals and Immunization.* Six to 7 week old dd/Y strain mice received intraperitoneal injection of 250 μg/0.2 mL LPS. SRBC (2×10^8 cells/0.2 mL) were injected from the tail vein. On days indicated five mice were sacrificed, and spleens were removed. Spleens were immediately frozen and kept at −80 °C until RNA extraction. LPS was prepared from *Escherichia coli* according to Westphal et al. (1952). SRBC was obtained commercially.

(b) *Preparation of RNA.* MOPC 41 L_κ chain mRNA was purified from myeloma as described before (Honjo et al., 1974). MOPC 31C L_κ chain mRNA was a generous gift of Dr. Ono (Ono et al., 1977a). To quantitate mRNA in spleen cells, RNA was extracted from cytoplasmic fraction of spleen by a slight modification of the method described by Kirby (1968). Spleens were homogenized with a Potter-type homogenizer (9 strokes) in 10 volumes of 0.25 M sucrose containing 50 mM Tris-HCl (pH 9.0), 25 mM KCl, 5 mM $Mg(CH_3COO)_2$, 0.5 mM cycloheximide, and 0.1% diethyl pyrocarbonate. The homogenate was centrifuged for 10 min at 5000*g* at 0 °C. To the supernatant was added sodium dodecyl sulfate to 0.3%. The mixture was extracted with an equal volume of phenol-*m*-cresol mixture (water-saturated phenol/*m*-cresol, 9:1, v/v) containing 0.1% 8-hydroxyquinoline. After centrifugation the aqueous phase was removed and extraction was repeated until no more white material was visible at the interphase. The RNA was precipitated with ethanol at −20 °C. The precipitated RNA was dissolved in H_2O and stored at −80 °C.

(c) *Preparation of cDNA.* L_κ chain cDNA was prepared with avian myeloblastosis virus reverse transcriptase (a gift from Dr. Leder) from L_κ chain mRNA purified from MOPC 41 myeloma (Honjo et al., 1974). L_λ chain cDNA was prepared from RPC 20 myeloma mRNA (Honjo et al., 1976a,b). Preparation of H_{γ_1} chain cDNA from MOPC 31C H_{γ_1} chain mRNA was described previously (Ono et al., 1977b). Globin cDNA was synthesized according to Ross et al. (1973). The specific radioactivity of cDNAs was 1×10^7 cpm/μg. L chain cDNAs, H_{γ_1} chain cDNA, and globin cDNA thus prepared were approximately 490, 600, and 400 nucleotides long, respectively, as measured by polyacrylamide gel electrophoresis in formamide (Honjo et al., 1976a). Most of the sequences of the Ig cDNAs employed in the present study are complementary to the constant region sequences which are common to all the Ig mRNAs of the respective subclasses (Honjo et al., 1974, 1976a,b; Ono et al., 1977b). Accordingly, hybridization to these cDNAs allows us to determine the total concentration of the Ig mRNAs which have a variety of variable region sequences.

(d) *Quantitation of mRNAs in Spleen RNA.* Increasing amounts of splenic RNA were hybridized to respective [^{3}H]cDNA in 0.6 M NaCl at 75 °C. Hybrids formed were assayed by S1 nuclease digestion as described before (Honjo et al., 1974). The initial hybridization rate (percentage hybridized per C_rt) was determined from the slope obtained as shown in Figure 1 (Marbaix et al., 1975; Orkin et al., 1975). Under the conditions employed the amount of hybridizable RNA is at least in fivefold excess over that of hybridized cDNA. The fraction of mRNA in total RNA is calculated from the ratio of the initial hybridization rate of spleen RNA to that of purified mRNA, assuming that the purified mRNA is 100% pure. The initial hybridization rates (hybridization percentage per C_rt) of MOPC 41 mRNA (L_κ), RPC 20 (L_λ), MOPC 31C mRNA (H_{γ_1}), and mouse globin mRNA are 1.66×10^5, 1.66×10^5, 1.38×10^5, and 2.4×10^5, respectively, under the same conditions. The average number of mRNA molecules in a spleen cell was calculated as follows: (total RNA content per cell) × (fraction of mRNA in total RNA) × 6.02×10^{23}/(molecular weight of mRNA). L_κ chain mRNA, L_λ chain mRNA, H_{γ_1} chain mRNA, and globin mRNA (α and β chains combined) are 4.5×10^5, 4.0×10^5, 7.0×10^5, and 4.2×10^5 daltons, respectively (Honjo et al., 1974, 1976a; Ono et al., 1977b).

(e) *Purification of B Lymphocytes from Mouse Spleen.* Spleen cells were suspended in Gey's solution (Gey and Gey, 1936) containing 0.08 M NH_4Cl for 2 min at 0 °C to lyse erythrocytes and erythroid cells. The residual cells were spun down and suspended in Eagle's minimal essential medium to make 1×10^7 spleen cells/mL. T lymphocytes were lysed by rabbit antiserum directed against mouse brain-associated T lymphocyte antigen (a gift from Dr. Okumura of Chiba University) and guinea pig complement by the method of Sato et al. (1976). B lymphocytes which survived the anti-T-lymphocyte serum treatment were spun down, washed several times by centrifugation, and frozen at −80 °C until RNA extraction. Alternatively, B lymphocytes were isolated by adsorption to nylon wool (Julius et al., 1973).

(f) *Determination of IgM- and IgG-Producing Cell Number.* LPS induces production of IgM against a variety of antigens. We have counted the number of spleen cells which produce IgM against SRBC as a measure of IgM synthesis in spleen. The indirect plaque assay was used to measure the sum of IgG- and IgM-producing cells (Šterzl and Říha, 1965; Dresser and Wortis, 1965). The number of IgG-producing cells was determined as the difference of the direct plaque count (IgM-producing cells) from the indirect plaque count. The number of plaque-forming cells was determined according to Cunningham (1965). The plaque numbers were counted in four slides which contain 10^5 to 10^6 cells for each time point and the average numbers were plotted.

(g) *Other Methods.* Thermal stabilities of the hybrids formed between spleen RNA and cDNA were tested by S1 nuclease digestion as described by Honjo et al. (1976b). The size of RNA was determined by centrifugation in a sucrose gradient (5 to 22%) as described before (Honjo et al., 1974). RNA was heated at 70 °C for 10 min and immediately chilled before centrifugation. DNA and RNA were determined colorimetrically according to Schneider (1957).

Results

(a) *Quantitation of L Chain mRNA Sequence in Spleen*

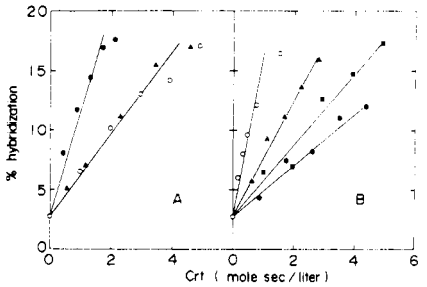

FIGURE 1: Hybridization of LPS-stimulated spleen RNA to L_κ chain cDNA. Increasing amounts of splenic RNA were hybridized to a constant amount of L_κ chain [^{3}H]cDNA as described in Experimental Procedures. Each reaction mixture (250 μL) contained 800 cpm of cDNA and was incubated for 30 min. Fractions of L_κ chain mRNA were calculated from slopes as described in Experimental Procedures and summarized in Table I. Symbols represents spleen RNA obtained on various days after LPS injection. (A) (○) Day 0; (▲) day 1; (●) day 2; (B) (○) day 3; (▲) day 5; (●) day 7; (■) day 10.

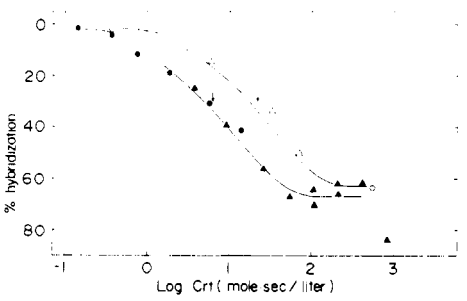

FIGURE 2: Hybridization kinetics of spleen RNA to L_κ chain cDNA. RNAs obtained from normal spleen and from LPS-stimulated spleen (day 3) were hybridized to L_κ chain cDNA. Aliquots were removed at time intervals and hybrids formed were assayed as described in Experimental Procedures. Spleen RNA used were the same batch as used in Figure 1. (○) Normal spleen RNA, 2.2 mg/mL; (△) 3.5 mg/mL of normal spleen RNA; (●) 0.56 mg/mL of LPS-stimulated spleen RNA; (▲) 5.4 mg/mL of LPS-stimulated spleen RNA.

RNA. Cytoplasmic RNA was extracted from mouse spleen at various time intervals after LPS injection and tested for its hybridizability to [^{3}H]cDNA complementary to the L_κ chain mRNA purified from a mouse myeloma, MOPC 41. Our assay scores all L_κ chain mRNA since RNA was hybridized to cDNA, 78% of whose sequence is complementary to the constant region sequence (see below). Figure 1 shows the initial rates of hybridization obtained from the plot of the extent of hybridization vs. C_rt. The sequence hybridizable to L_κ chain cDNA began to increase 2 days after LPS injection, reaching a maximum (4.4-fold) on day 3. The hybridizable sequence decreased rather quickly, going back to the normal level by day 7. The results indicate that the L_κ chain mRNA sequence accumulated temporarily in mouse spleen by LPS injection. The concentration of L_κ chain mRNA sequence increased similarly in total cellular RNA extracted from LPS-treated spleen.

(b) *Characterization of L_κ Chain mRNA Sequence in Spleen.* Hybridization kinetics between L_κ chain cDNA and RNA derived from day 0 and day 3 spleens gave $C_rt_{1/2}$ values of 26 and 6.3, respectively, as shown in Figure 2. Since the $C_rt_{1/2}$ value is inversely proportional to the concentration of the sequence, the ratio of the $C_rt_{1/2}$ values indicates that 4.1-fold greater concentration of the L_κ chain mRNA sequence is present in day 3 spleen RNA than in day 0 spleen RNA, which is in good agreement with the results measured by the initial rate of hybridization as shown above. The maximal extent of hybridization reached 64% to 67% with RNAs from day 0 and day 3 spleens. The extents of hybridization likely

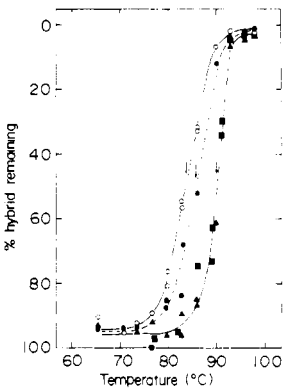

FIGURE 3: Thermal stability of hybrids formed between L_κ chain cDNA and spleen RNA. L_κ chain cDNA was hybridized with normal spleen RNA, LPS-stimulated spleen RNA (day 3), and L_κ chain mRNAs purified from MOPC 41 and MOPC 31C myelomas until C_rt values of 470, 450, and 0.2, respectively, were attained. The hybrids formed were tested for their thermal stability as described in Experimental Procedures. T_m values for hybrids with normal spleen RNA, LPS-stimulated spleen RNA, MOPC 41 mRNA, and MOPC 31C mRNA were 84, 86, 90, and 90 °C, respectively. (○) Normal spleen RNA; (●) LPS-stimulated spleen RNA; (▲) MOPC 41 mRNA; (■) MOPC 31C mRNA.

reflect that proportion of the cDNA complementary to the L_κ chain constant region sequence (Honjo et al., 1974). Hybridization with L_κ chain mRNAs purified from myelomas, MOPC 41 (homologous) and MOPC 31C (heterologous), attained the maximal extent of 73% and 65% (data not shown), respectively, indicating that 78% of the hybridizable sequence of the cDNA preparation employed represents the constant region sequence. Figure 3 shows the thermal stability of the hybrids. The melting profiles of the hybrids were reasonably sharp and T_m values for the hybrids with normal spleen RNA, LPS-stimulated spleen RNA, and purified L_κ chain mRNAs (both MOPC 41 and MOPC 31C mRNAs) were 84, 86, and 90 °C, respectively. The results indicate that most of the sequences in the spleen RNA hybrids are congruently base paired. Nonetheless, a slight reduction of the T_m values for the splenic RNAs compared with those of the purified L_κ chain mRNAs could be due to partially inaccurate base pairing in the variable region and/or untranslated sequences which constitute approximately 20% of the cDNA sequence (Honjo et al., 1974).

When the RNA fractions from normal as well as LPS-stimulated spleen were treated with RNase or alkali, hybridization with the L_κ chain cDNA was completely abolished, indicating that the sequence assayed is present in RNA molecules. Heat denaturation of normal spleen RNA did not increase the amount of the sequence hybridizable to L_κ chain cDNA. This excludes the possibility that a double-stranded "precursor" RNA exists in normal spleen and is converted to a hybridizable form by the LPS stimulation. The sequence hybridizable to the L_κ chain cDNA from both normal and LPS-stimulated spleens sedimented with a major peak at 13 S upon centrifugation in sucrose gradient under the conditions which abolish nonspecific aggregation of RNA (Honjo et al., 1974), although some degradation of RNA was inevitable. The sedimentation profile of myeloma L chain mRNA shows a single peak at 13–14 S (Honjo et al., 1976a). The L_κ chain mRNA sequences in splenic RNA were adsorbed to oligo(dT)-cellulose, suggesting that they contain poly(A) sequences.

(c) *L_κ Chain mRNA Accumulation and IgM Synthesis.* Intraperitoneal injection of LPS brings about a variety of biochemical as well as cytological changes in mouse spleen

TABLE I: Quantitation of L_κ Chain mRNA in LPS-Stimulated Spleen.

Days after LPS injection	(Cells[a]/ spleen) $\times 10^{-8}$	RNA/ cell (pg)	RNA/ spleen (mg)	Initial rate of hybridization[b]	(mRNA/ total RNA) $\times 10^5$	mRNA molecules/ cell	(mRNA molecules/ spleen) $\times 10^{-10}$
0	3.1	2.11	0.65	3.15	1.90	53	1.65
1	2.5	4.19	1.06	3.15	1.90	105	2.63
2	3.6	3.98	1.44	9.20	5.54	292	10.5
3	5.8	3.05	1.76	14.0	8.43	340	19.7
5	4.7	3.07	1.45	4.85	2.92	119	5.58
7	7.9	2.89	2.28	2.15	1.30	50	3.93
10	5.6	2.37	1.39	3.00	1.81	57	3.18

[a] Cell numbers were calculated from DNA contents assuming that a mouse spleen cell contains 6 pg DNA. [b] Initial rates of hybridization were expressed as percentage hybridized per C_rt which was calculated from Figure 1.

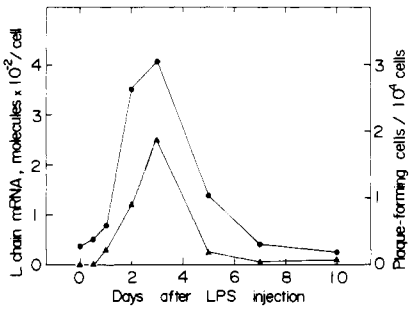

FIGURE 4: Effects of LPS injection on spleen cells. IgM-producing cell numbers were determined as described in Experimental Procedures. The number of L_κ chain mRNA molecules per spleen cell were determined as described in Figure 1. (●) Average number of L_κ chain mRNA molecules in a spleen cell; (▲) number of plaque-forming cells against SRBC.

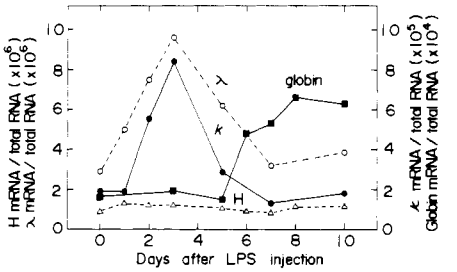

FIGURE 5: Kinetics of Ig mRNA accumulation in LPS-stimulated spleen. Fraction of each mRNA was determined by hybridization to a constant amount of respective cDNA exactly as described in Figure 1 and Experimental Procedures. Data for L_κ chain mRNA were taken from Table II. Spleen RNA used was the same batch as used in Figure 1 and Table II. (●) L_κ chain mRNA; (○) L_λ chain mRNA; (△) H_{γ_1} chain mRNA; (■) globin mRNA.

(Gronowicz and Coutinho, 1974; Takano et al., 1967; Fruhman, 1966). Consistent with previous reports from other laboratories (Coutinho and Möller, 1975) that both DNA and RNA syntheses are stimulated by LPS injection, the number of cells per spleen almost doubled on day 3 and the amount of RNA per spleen increased by 2.7-fold as shown in Table I. Assuming that the purified L_κ chain mRNA is 100% pure we can calculate the average number of L_κ chain mRNA molecules present in a spleen cell or in a spleen. On day 3 the fraction of L_κ chain mRNA, the average number of L_κ chain mRNA molecules per cell, and the average number of L_κ chain mRNA molecules per spleen were elevated by 4.4-fold, 6.4-fold, and 11.9-fold, respectively. IgM synthesis as measured by the number of plaque-forming cells against SRBC increased and reached a maximal level on day 3, followed by a rapid disappearance by day 7 as shown in Figure 4. It is clear that the accumulation of L_κ chain mRNA parallels the increase of IgM synthesis.

(d) *Specific Accumulation of L Chain mRNA.* In order to see whether the accumulation of L_κ chain mRNA is specific we have measured the concentration of several other mRNAs in LPS-stimulated spleen. Figure 5 shows such studies on L_λ chain mRNA, H_{γ_1} chain mRNA and globin mRNA. The fractional content of L_λ chain mRNA, as expected, increased by 3.3-fold on day 3 though the concentration of L_λ chain mRNA is one order of magnitude less than that of L_κ chain mRNA. L_λ chain mRNA seems to accumulate and disappear in good coincidence with L_κ chain mRNA. On the other hand, the concentration of H_{γ_1} chain mRNA remained essentially constant. The results are consistent with the reports from other laboratories that a single injection of LPS does not induce IgG synthesis in vivo (Britton and Möller, 1968; Coutinho and Möller, 1975). The globin mRNA concentration remained constant until day 5. On day 6, however, a significant increase (3-fold) of the mRNA concentration was seen which was conserved until day 10. The results confirm the report that LPS stimulates the erythropoietic activity in mouse spleen (Fruhman, 1966).

TABLE II: Accumulation of L_κ Chain mRNA in LPS-Stimulated B Lymphocytes.

Method of B-lymphocyte isolation[a]	Fraction of L_κ chain mRNA ($\times 10^5$)	
	Control	LPS (day 3)
Nylon wool	4.92	17.5
anti-T-lymphocyte serum	1.02	6.50

[a] Methods of B-lymphocyte isolation were described in Experimental Procedures.

(e) *L_κ Chain mRNA Content in Purified B Lymphocytes.* We have measured the L_κ chain mRNA concentration in B lymphocytes which were purified from normal and LPS-stimulated spleen cells by adsorption to nylon wool or by the treatment with rabbit antiserum directed against mouse T lymphocytes. Table II shows that the L_κ chain mRNA concentration increased by 3.6- to 6.3-fold in B lymphocytes derived from LPS-stimulated spleen. The L_κ chain mRNA concentrations in normal and LPS-stimulated B lymphocytes were comparable to those in normal and LPS-stimulated spleen cells, respectively. Since about 50% of spleen cells are B lymphocytes, they seem to contain a major portion, if not all, of L_κ chain mRNA in spleen.

TABLE III: Quantitation of L_κ Chain mRNA in SRBC-Stimulated Spleen.

Days after SRBC injection	(Cells[a]/ spleen) $\times 10^{-8}$	RNA/ cell (pg)	RNA/ spleen (mg)	Initial rate of hybridization[b]	(mRNA/ total RNA) $\times 10^5$	mRNA molecules/ cell	(mRNA molecules/ spleen) $\times 10^{-10}$
0	4.3	1.58	0.679	2.04	1.23	26	1.11
1	4.0	2.05	0.820	2.78	1.67	48	1.91
2	4.0	1.70	0.680	2.70	1.63	37	1.47
3	4.0	2.71	1.08	2.88	1.73	62	2.48
5	4.0	2.35	0.940	3.53	2.13	66	2.65
7	3.8	1.89	0.718	5.23	3.15	79	2.99
10	4.1	1.88	0.771	5.97	3.60	90	3.67

[a] Cell numbers were calculated from DNA contents assuming that a mouse cell contains 6 pg of DNA. [b] Initial hybridization rates were expressed as percentage hybridized per C_0t which was determined as described in Figure 1.

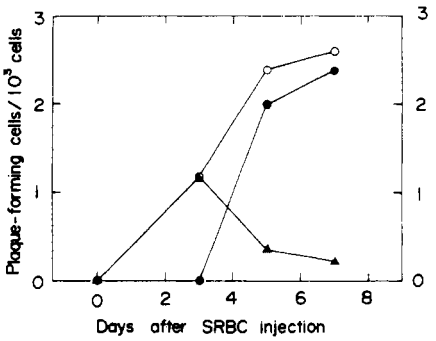

FIGURE 6: Increase of IgM- and IgG-producing cells in SRBC-stimulated spleen. Numbers of IgM- and IgG-producing cells were determined as described in Experimental Procedures at time intervals indicated after SRBC injection. (○) Total number of plaque-forming cells; (▲) number of IgM-producing cells; (●) number of IgG-producing cells.

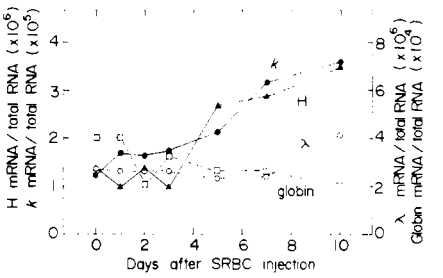

FIGURE 7: Kinetics of Ig mRNA accumulation in SRBC-stimulated spleen. Fraction of each mRNA was determined by hybridization to a constant amount of respective cDNA exactly as described in Figure 1 and Experimental Procedures. The same batch of spleen RNA was used throughout the experiments. (●) L_κ chain mRNA; (○) L_λ chain mRNA; (▲) $H_{\gamma 1}$ chain mRNA; (□) globin mRNA.

(f) *Ig mRNA Accumulation in SRBC-Stimulated Spleen.* It is important to see whether a normal antigen also induces accumulation of Ig mRNA or not. We have used SRBC which is known as a potent antigen. As shown in Figure 6, SRBC stimulates IgM synthesis in mouse spleen within 2–3 days after its injection, followed by extensive IgG synthesis around day 5 to 7 in confirmation of the results reported by Dresser and Wortis (1965) and Šterzl and Říha (1965). Table III shows the kinetics of the spleen cell number, RNA content, and the L_κ chain mRNA concentration in SRBC-stimulated spleen. In contrast to LPS stimulation, SRBC injection did not increase the number of spleen cells. The RNA content was slightly elevated (1.6-fold per spleen at most). The fractional mRNA content rose slightly on day 1 and then increased significantly on day 5, reaching a 2.9-fold higher level on day 10. Similar studies on L_λ chain mRNA, $H_{\gamma 1}$ chain mRNA, and globin mRNA are shown in Figure 7. The concentration of L_λ chain mRNA maintained the original level until day 7 and increased slightly (1.5-fold) on day 10. The $H_{\gamma 1}$ chain mRNA concentration began to increase extensively on day 5, reaching a 2.5-fold higher level on day 10. The results are in agreement with the results that SRBC injection induces IgG synthesis in a later stage (days 5–7) of the immune response (Figure 6). There was no increment of the globin mRNA content throughout 10 days after SRBC injection. These results show that an ordinary antigen like SRBC also induces the specific accumulation of Ig mRNAs concurrently to the increase of IgG synthesis.

Discussion

The final step of the immune response, which is manifested as induction of Ig synthesis in B lymphocytes, might be controlled in terms of amplification of the Ig genes or in terms of inducing their transcription or translation of the corresponding mRNAs. The genes coding for L chain mRNA have been shown to be repeated not more than five times in the mouse genome regardless of whether the cells from which DNA was obtained are actively engaged in Ig synthesis or not (Honjo et al., 1974, 1976b; Stavnezer et al., 1974; Faust et al., 1974). The results rule out the possibility that gene amplification plays an essential role for the immune response.

We have shown accumulation of Ig mRNAs in mouse spleen cells as they divide and differentiate into high-rate antibody-secreting lymphocytes in response to LPS or SRBC injection. Since the concentration of L_κ chain mRNA increases in the total cellular RNA including nuclear as well as cytoplasmic RNA, it is unlikely that the accumulation of Ig mRNA is attributable to the increase of nucleocytoplasmic transport. The apparent accumulation of Ig mRNAs in spleen could be also due to an increase of the B-lymphocyte population in a spleen while each B lymphocyte contains the unvaried amounts of Ig mRNAs. The above possibility was excluded by the experiment in which purified B lymphocytes were shown to contain an elevated amount of L_κ chain mRNA per cell upon LPS stimulation (Table II). Furthermore, the quantitative consideration makes the above possibility unlikely. Shands et al. (1973) have reported that about 50% of spleen cells are activated by LPS stimulation. Since approximately 50% of spleen cells are B lymphocytes in normal spleen (Raff et al., 1971; Storb et al., 1976), LPS seems to stimulate a majority of B lymphocytes in spleen. Therefore, the enlargement of the B-lymphocyte population within a spleen cannot increase the average Ig mRNA content per spleen cell more than twice as much as the normal level.

Since the time course of the accumulation and disappear-

ance of the L chain mRNA agrees with kinetics of IgM synthesis, the number of mRNA molecules present seems to be closely related with the Ig synthetic capacity of lymphocytes. This implies that regulation involving the translational activation of a reserve population of L chain mRNA does not seem to play a major role in induction of Ig synthesis. Although our data do not allow us to conclude whether the accumulation of L chain mRNA is attributable to the increase of the Ig gene transcription or to the reduction of mRNA degradation, preliminary studies which have been undertaken in in vitro cultured spleen cells show that transcription of the L_κ chain gene is enhanced by LPS stimulation (Tsuda, Natori, and Honjo, manuscript in preparation).

It is apparent that there are quantitative as well as qualitative similarities between L_κ chain mRNA from spleen and from mouse myelomas. Both mRNAs have poly(A) sequences and a similar size. The extent of hybridization of splenic RNA to MOPC 41 cDNA is similar to that of heterologous L_κ chain mRNA purified from MOPC 31C myeloma. The hybrids with splenic mRNA have the T_m values slightly lower than myeloma mRNA hybrids. The difference of the T_m values (4 to 6 °C) corresponds to a maximum of 4 to 6% mismatching (Britten et al., 1974). It is plausible that partially mismatched hybrids are formed between MOPC 41 cDNA and splenic mRNA, which is a mixture of hundreds of L_κ chain mRNA species, each containing different variable region sequences. In fact, the extent of hybridization of splenic mRNA is similar to, nonetheless, usually slightly higher than that of the heterologous L_κ chain mRNA. The decreased thermal stability of the hybrids could be ascribed to several other factors such as length of hybridizing RNA and/or presence of L chain-like RNA of unknown function. Storb et al. (1976) reported that the L_κ chain cDNA hybrid with spleen RNA melts 10.7 °C below the T_m value of the hybrid with homologous mRNA.

The kinetics of Ig mRNA accumulation agree with those immunological observations which have been known to be induced by LPS or SRBC injection. LPS injection induces accumulation of L chain mRNA but not of $H_{\gamma 1}$ chain mRNA, corresponding to stimulation of IgM synthesis but not of IgG synthesis. SRBC-induced accumulation of L chain mRNA and $H_{\gamma 1}$ chain mRNA coincides with IgG synthesis. It was rather unexpected that SRBC injection did not increase L chain mRNAs significantly at an early stage (days 2-3) when spleen cells produce IgM. This is probably due to the fact that SRBC injection induces much smaller quantities of IgM synthesis than does LPS injection. The increase of the globin mRNA level coincides with the report that LPS injection stimulates erythropoiesis in a week or so in mouse spleen (Fruhman, 1966). Our preliminary data show that LPS does not stimulate the erythropoietic activity in cultured spleen cells (Tsuda, Natori, and Honjo, manuscript in preparation), suggesting that LPS-induced erythropoiesis in spleen is a secondary effect of the bacterial endotoxin.

Investigators from another laboratory reported the quantitation of Ig H chain mRNA in LPS-stimulated mouse spleen cells cultured in vitro (Stevens et al., 1975). The assay method employed for mRNA measurement, however, was shown to be difficult to reproduce (Stevens and Williamson, 1975).

Acknowledgments

We thank Dr. D. Mizuno for his support and encouragement and Dr. P. Leder for a generous gift of reverse transcriptase. The manuscript was improved by the critical reading by Drs. P. Leder and R. Crouch. Thanks are also extended to Drs. K. Okumura and T. Tada for helpful advice and suggestions.

References

Britten, R. J., Graham, D. E., and Neufeld, B. R. (1974), *Methods Enzymol. 29,* 363-418.

Britton, S., and Möller, G. (1968), *J. Immunol. 100,* 1326-1334.

Coutinho, A., and Möller, G. (1975), *Adv. Immunol. 21,* 113-238.

Cunningham, A. J. (1965), *Nature (London) 207,* 1106-1107.

Dresser, D. W., and Wortis, H. H. (1965), *Nature (London) 208,* 859-861.

Faust, C. H., Diggelmann, H., and Mach, B. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 2491-2495.

Fruhman, G. J. (1966), *Blood 27,* 363-370.

Gey, G. O., and Gey, M. K. (1936), *Am. J. Cancer 27,* 45-76.

Gronowicz, E., and Coutinho, A. (1974), *Eur. J. Immunol. 4,* 771-776.

Honjo, T., Packman, S., Swan, D., and Leder, P. (1976b), *Biochemistry 15,* 2780-2785.

Honjo, T., Packman, S., Swan, D., Nau, M., and Leder, P. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3659-3663.

Honjo, T., Swan, D., Nau, M., Norman, B., Packman, S., Polsky, F., and Leder, P. (1976a), *Biochemistry 15,* 2775-2779.

Julius, M. H., Simpson, E., and Herzenberg, L. A. (1973), *Eur. J. Immunol. 3,* 645-649.

Kirby, K. S. (1968), *Methods Enzymol. 12B,* 87-99.

Marbaix, G., Huez, G., Burny, A., Cleuter, Y., Hubert, E., Leclercq, M., Chantrenne, H., Soreq, H., Nudel, U., and Littauer, U. Z. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 3065-3067.

Ono, M., Kondo, T., and Kawakami, M. (1977a), *J. Biochem. (Tokyo) 81,* 941-947.

Ono, M., Kondo, T., Kawakami, M., and Honjo, T. (1977b), *J. Biochem. (Tokyo) 81,* 949-954.

Orkin, S. H., Swan, D., and Leder, P. (1975), *J. Biol. Chem. 250,* 8753-8760.

Rabbitts, T. H. (1974), *FEBS Lett. 42,* 323-326.

Raff, M. C., Nase, S., and Mitchison, N. A. (1971), *Nature (London) 230,* 50-51.

Ross, J., Aviv, H., and Leder, P. (1973), *Arch. Biochem. Biophys. 158,* 494-502.

Sato, V. L., Waksal, S. D., and Herzenberg, L. A. (1976), *Cell. Immunol. 24,* 173-185.

Schechter, I. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2511-2514.

Schneider, W. C. (1957), *Methods Enzymol. 3,* 680-684.

Shands, J. W., Jr., Peavy, D. L., and Smith, R. T. (1973), *Am. J. Pathol. 70,* 1-24.

Stavnezer, J., Huang, R. C., Stavnezer, E., and Bishop, J. M. (1974), *J. Mol. Biol. 88,* 43-63.

Šterzl, J., and Říha, I. (1965), *Nature (London) 208,* 858-859.

Stevens, R. H., Askonas, B. A., and Welstead, J. L. (1975), *Eur. J. Immunol. 5,* 47-54.

Stevens, R. H., and Williamson, A. R. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4679.

Storb, U., Hager, L., Putnam, D., Buck, L., Fabin, F., and Clagett, J. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 2467-2471.

Takano, T., Yoshioka, O., and Mizuno, D. (1967), *Proc. Jpn. Soc. RES 7,* 13-18.

Westphal, O., Lüderitz, O., and Bister, F. (1952), *Z. Naturforsch. B 7,* 148-155.