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RESPIRATORY SYNCYTIAL VIRUS INFECTION IN SYRIAN HAMSTERS. II. REGULATION OF THE IMMUNE RESPONSE

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Summary. — The dynamics of immune cell responsiveness in the process of development of primary and secondary immune responses after infection of adult Syrian hamsters with native respiratory syncytial virus (RSV) was studied. In primary immune response, the enhancement of functional responsiveness of lymphocytes to mitogens and the maximum level of their spontaneous proliferative activity were found to preceed the optimal level of antibody synthesis. Regulation of the dynamics of immunogenesis after reinfection of animals showed typical features of the secondary immune response.

Key words: RS virus; immune response; suppressor-helper activity

Introduction

Since immune response is associated with development of specific and non-specific protection, the regulation of immunogenesis depends not only on specific mechanisms but is also determined by non-specific general and cellular resistance to viruses. Investigations of the mechanisms of immune response regulation including the assessment of the helper-suppressor activity of lymphocytes are still particular, being focussed on the patterns of protection in viral infections (Liew and Russell, 1980; Russell and Liew, 1980). However, it has already become necessary, to investigate the dynamics of immune response with special reference to the manifestations of helper, effectory, and suppressor activities of lymphocytes which create prerequisites for the correction of appropriate reactions.

The elucidation of the patterns of immune response to a certain agent requires a reproducible experimental model of viral infections important in human pathology. In this respect, marked features of RSV infection may be of interest (Calvet, 1980). Our first paper described the pathogenesis of the acute period of RSV infection in adult Syrian hamsters and the dynamics of development of humoral immunity (Skovikova *et al.*, 1983). This paper presents the results on cellular reaction of lymphocytes in the course of primary and secondary immune response after infection and reinfection of adult Syrian hamsters with native RSV.

Materials and Methods

The conditions of animal infection and the analysis of humoral response were described in previous paper (Skovikova *et al.*, 1983).

Spleens of individual hamsters were used for preparation of splenocytes from at least 3—5 animals at each interval. The cells obtained by mild homogenization were propagated in a concentration of 6×10^6 in 2 ml of medium 199 supplemented with 5 mmol/l glutamine (Reanal, Hungary), 10^{-8} mmol/l β-mercaptoethanol (Serva, U.S.A.), 400 IU/l insulin, 6.7 mmol/l glucose, 5% foetal calf serum (The N. F. Gamaleya Institute of Epidemiology and Microbiology, U.S.S.R. Acad. Med. Sci.) in 5% CO_2 atmosphere (Meshcheryakova *et al.*, 1979). The viability of cells during propagation was assessed by vital staining with trypan blue.

Spontaneous and mitogen-induced blastic transformation of splenocytes was assessed radiometrically. The level of DNA synthesis in 72-hr cultures was determined by incorporation of ^{3}H-thymidine (specific activity 555 GBq/mmol, "Isotope", U.S.S.R.). The labelled precursor was added in a dose of 74 kBq in 0.1 ml per culture 18 hr before termination of incubation. The uptake of the label into the acid-insoluble fraction was measured in a scintillation counter Isocap-300 (Nuclear Chicago, U.S.A.). The mitogens used were a high molecular fraction of *S. paratyphi* B, strain 42 lipopolysaccharide (LPS) (Research Institute of Vaccines and Sera, Leningrad) and phytohaemagglutinin P (PHA (Difco, U.S.A.). The mitogens were added in 0.1 volumes containing 50 μg LPS and PHA in a dilution of 1 : 32 per culture, respectively.

The results were expressed as a stimulation index (SI) which was a ratio of the level of ^{3}H--thymidine incorporation by mitogen-stimulated cells to that in non-stimulated splenocytes.

The validity of the changes observed was evaluated by the variation statistic method using Student's t criterion.

Results

The dynamics of proliferative activity of splenocytes from RSV-infected hamsters assessed by spontaneous thymidine incorporation was compared with that in intact animals. There was a significant increase in thymidine

Table 1. Spontaneous and mitogen-induced ^{3}H-thymidine incorporation to splenocytes of adult Syrian hamsters during RSV infection

Time	DNA synthesis (c.p.m.)	P	Index of PHA-stimulation	P	Index of LPS-stimulation	P
Before infection	170 ± 20		0.9 ± 0.1		0.8 ± 0.1	
After infection:						
Day 1	385 ± 30	< 0.05	8.2 ± 0.2	< 0.001	14.6 ± 0.3	< 0.0001
Day 3	730 ± 50	< 0.001	1.1 ± 0.1	> 0.05	0.8 ± 0.1	> 0.05
Day 5	450 ± 35	< 0.05	0.4 ± 0.1	> 0.05	1.1 ± 0.1	> 0.05
Day 7	250 ± 60	> 0.05	2.0 ± 0.1	< 0.05	4.1 ± 0.1	< 0.001
Day 14	380 ± 30	< 0.05	0.5 ± 0.1	> 0.05	2.1 ± 0.1	< 0.05
Day 20	240 ± 50	> 0.05	0.9 ± 0.1	> 0.05	0.7 ± 0.1	> 0.05
Day 30	220 ± 30	> 0.05	0.5 ± 0.1	> 0.05	0.8 ± 0.1	> 0.05
After reinfection:						
Day 1	760 ± 25	< 0.001	2.2 ± 0.1	< 0.05	7.6 ± 0.2	< 0.0001
Day 5	520 ± 35	< 0.001	0.6 ± 0.1	> 0.05	1.5 ± 0.1	> 0.05

incorporation as early as the 1st day post-infection (p.i.) with the maximum blastogenesis by 3 days (see Table 1). A sharp decline in proliferation of splenocytes occurred on day 7 with another significant increase on day 14 p.i. Since day 20 p.i., the DNA-synthesizing activity of splenocytes has returned to normal.

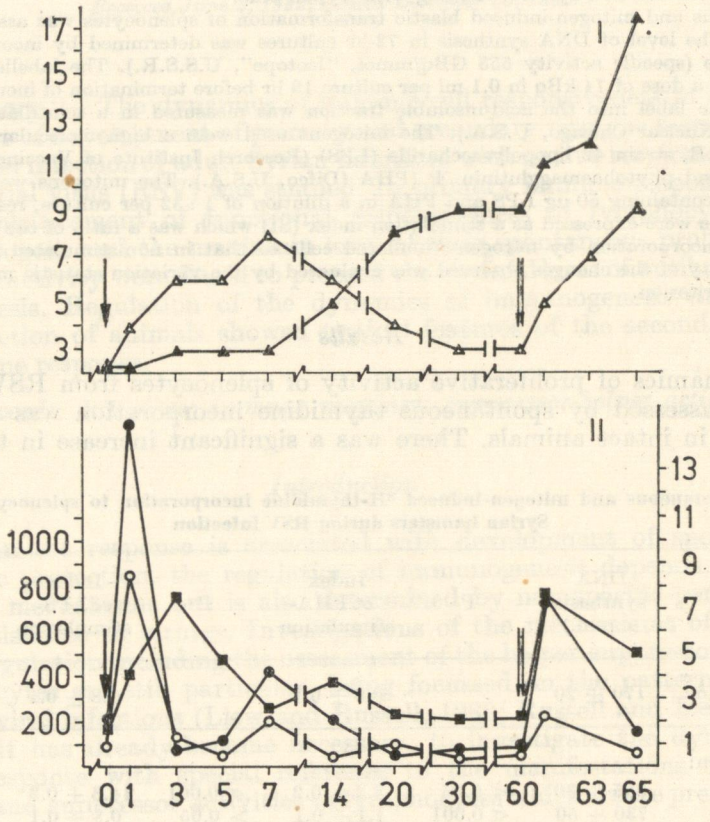

Fig. 1.

The synthesis of secretory and serum antibodies and cell-mediated reactions of lymphocytes in RSV infected adult Syrian hamsters

I: Ordinate: antibody titres (in $\log_2$)

△ — titres of secretory antibodies; ▲ — titres of serum antibodies.

II: Left ordinate: spontaneous incorporation of ^{3}H-thymidine (c.p.m.); right ordinate: lymphocyte stimulation index by PHA and LPS; abscissa — days p.i.

●—— ● SI by LPS

○—— ○SI by PHA

■—— ■ spontaneous ^{3}H-thymidine incorporation

↓ — primoinfection; ↓ ↓ — reinfection.

Upon reinfection, a rapid and significant increase of blastogenesis occurred within 24 hr. The study on the functional responsiveness of splenocytes of intact hamsters showed this group of lymphocytes to be resistant to the effect of mitogens. No stimulation of the proliferative activity of splenocytes of intact animals could be achieved during 72 hr of their propagation in the presence of the standard PHA and LPS concentrations (Table 1). Checking of the same mitogen lots in splenocytes from C57 Black/6 mice showed the SI of splenocytes for PHA was about 7.5 and for LPS exceeded 100. During development of RSV infection, hamster lymphocytes became liable to stimulation by these mitogens. Significant changes in the proliferative activity of splenocytes under the effect of the mitogens showed a certain dynamics and coincided in time for PHA and LPS. Increased SI of splenocytes from infected animals after primary infection was recorded on day 1 and day 7 (Table 1). The LPS-induced blastic transformation of splenocytes persisted till 14 days p.i. After reinfection, the response to PHA and LPS increased significantly within the first 24 hr.

Comparison of the values of the cellular and humoral immunity revealed the following patterns (Fig. 1). A titre of secretory antibodies detectable since the first day of infection reached the peak on day 7. This has been preceded by an enhancement of the functional responsiveness of splenocytes in the presence of mitogens observed within the first 24 hr and reaching of the peak of spontaneous proliferative activity of splenocytes on day 3. A significant increase in titres of serum antibody since day 14 p.i. was preceded by a second peak of the functional responsiveness of splenocytes in the in the presence of mitogens on day 7 as well as by an another rise of spontaneous DNA-synthesizing activity of splenocytes on day 14; the second enhanced response to mitogens corresponded to the decrease of their spontaneous proliferative activity.

Reinfection of the animals was accompanied not only by a marked enhancement of blastogenesis and increase of the functional responsiveness of splenocytes to mitogens on day 1 but by a sharp simultaneous rise in the levels of secretory and serum antibodies reaching the peak on day 5. Thus, a certain sequence of events has been observed in the dynamics of the immune response: initially the functional responsiveness of immunocytes increased in the presence of mitogens followed by a maximum level of spontanenous proliferative activity of splenocytes after which antibody synthesis reached its optimum level.

Discussion

The mechanism of regulation of immune response in the establishment of antiviral immunity have been poorly studied yet. The regulation of immunogenesis in viral infections may be associated with the patterns of interaction of different viruses as active intracellular parasites with the cells of immune system (Dubrovina *et al.*, 1980; Polyak *et al.*, 1980).

The present study was aimed at the elucidation of the dynamics of immunogenesis regulation in RS virus infection of adult Syrian hamsters. For this

purpose, characteristics of the DNA-synthesizing activity of the total pool of spleen cells and the functional responsiveness of the T cell (PHA-response) and B cell (LPS-response) lymphocyte subpopulations were compared with development of humoral immunity. The enhanced functional responsiveness of lymphocytes in the presence of mitogens and increasing levels of spontaneous proliferative activity to the maximum were found to precede the optimum antibody synthesis.

LPS, a mitogen of mouse B lymphocytes, is known to achieve the stimulating effect within 72 hr of cell propagation (Anderson *et al.*, 1972; Gery *et al.*, 1972). This period of cell propagation is absolutely insufficient for the manifestation of LPS mitogenic effect for human lymphocytes (Peavy *et al.*, 1970). Human lymphocyte blastogenesis under the effect of LPS occurs only within 7—9 days. The effect of LPS is directed to B lymphocytes, however, in the presence of T cells the response of B lymphocytes to LPS stimulation markedly increases (Miller *et al.*, 1978). This auxillary function is provided by the subpopulation of helper T cells. Experiments in vitro showed that preliminary induction of the helper activity of T cells by suboptimal doses of the mitogen facilitated the blastogenic response of B lymphocytes to LPS (Lopatin *et al.*, 1980). High stimulation level of human B lymphocyte mitogenesis was achieved after a short period of cultivation under conditions of preliminary induction of the helper activity of T lymphocytes.

The foregoing proved to be quite valid for the evaluation of the functional responsiveness of the helper population of lymphocytes of adult Syrian hamsters, as demonstrated also by the time coincidence in detection of B cell blastogenesis and marked enhancement of T cell response to PHA. By this way the dynamics of the helper activity could have been followed indirectly. The dynamics of immune response regulation in acute RSV infection in adult Syrian hamsters underwent the following stages: on day 1 p.i. the helper activity increased, on day 3 marked blastogenesis of splenocytes reached its peak. This was accompanied by appearance of an antigen-specific clone of plasmocytes and achievement of the maximum level of secretory antibodies within 5—7 days. Another peak of the helper activity observed at 7 days p.i. was followed by increased proliferation of splenocytes at 14 days and appeared to be associated with production of a considerable level of serum antibodies. Noteworthy was also the time coincidence of the second peak of helper activity with the decline in DNA-synthesizing activity of splenocytes. The decrease in the proliferative activity of splenocytes may be explained by activation of the function of the suppressor cells at 3—5 days p.i. A certain, "acceptable" level of DNA synthesis in cells is presumed to be one of the signals triggering suppressor T cells (Gershon *et al.*, 1972). The activation of the suppressors could inhibit the excessive DNA synthesis leading to decreased cell proliferation by 5—7 days p.i.

The regulation of immunogenesis upon reinfection of the animals showed the typical features of secondary immune response. The study revealed the dynamics of regulation of immunogenesis in the course of development of the primary and secondary immune response after infection of adult Syrian hamsters with native RSV.

References

Anderson, J., Möller, G., and Sjöberg, O. (1972): Selective induction of DNA synthesis in T and B lymphocytes. *Cell Immunol.* **4**, 381—393.

Calvet, A. (1980): Que est-ce que le virus respiratoire syncytial? *Can J. med. Sci.* **5**, 1833—1834.

Dubrovina, T. Ya., Leontieva, G. F., Meshcheryakova, I. E., Polyak, R. Ya., and Smorodintsev, A. A. (1980): Features of interaction of influenza virus and macrophages in realization of immune response (in Russian). *Bull. exp. Biol. Med.* **8**, 192—194.

Gershon, R. K., Cohen, P., Hencin, R., and Leibhaber, S. A. (1972): Suppressor T cells. *J. Immunol.* **108**, 586—590.

Gery, I., Krueger, K., and Spiesel, S. L. (1972): Stimulation of B lymphocytes by endotoxin. *J. Immunol.* **118**, 2004—2008.

Liew, F. J., and Russel, S. M. (1980): Delayed type hypersensitivity to influenza virus. Induction of antigen-specific suppressor T cells for delayed type hypersensitivity to hemagglutinin during influenza virus infection in mice. *J. exp. Med.* **151**, 799—814.

Lopatin, D. E., Mangan. D. F., Horner, I. S., and Peebles, F. L. (1980): Mitogen-induced amplification of blastogenesis in lipopolysaccharide-precultures lymphocytes. *Infect. Immun.* **29**, 512—519.

Meshcheryakova, I. E., Surkova, E. A., and Polyak, R. Ya. (1979): Transformation of phytohemagglutinin-stimulated mouse lymphocytes in relation of the conditions of their cultivation (in Russian). *Tsitologiya* **21**, 982—985.

Miller, R. A., Gartner, S., and Kaplan, H. S. (1978): Stimulation of mitogenic responses in human peripheral blood lymphocytes by lipopolysaccharide: serum and T helper cell requirements. *J. Immunol.* **121**, 2160—2164.

Peavy, D. L., Adler, W. H., and Smith, R. T. (1970): The mitogenic effects of endotoxin and staphylococcal enterotoxin B on mouse spleen cells and human peripheral lymphocytes. *J. Immunol.* **105**, 1451—1458.

Polyak, R. Ya., Dubrovina, T. Ya., Leontieva, G. F., and Smorodintsev, A. A. (1980): Analysis of the mechanisms of resistance of the immune system cells to influenza virus (in Russian). *Dokl. Akad. Nauk SSSR* **251**, 725—728.

Russell, S. M., and Liew, F. J. (1980): Cell cooperation in antibody responses to influenza virus. I. Priming of helper T cells by internal components of the virion. *Eur. J. Immunol.* **10**, 791—796.

Skovikova, L. P., Taros, L. Yu., and Volgarev, A. P. (1983): Respiratory syncytial virus infecon in Syrian hamsters. I. Development of humoral immunity. *Acta virol.* **27**, 490—495.