PRIMATOLOGY

Parameters of the Cytokine System Functioning in Laboratory Primates

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The cytokine status (IFN, IL, *etc.*) of different monkey species (*M. mulatta*, *P. hamadryas*, *C. aethiops*) was studied. The interferon status is determined by the following parameters: IFN content in circulating blood and production of IFN-α and IFN-γ by lymphocytes after appropriate *in vitro* induction. The interferon status of monkeys is similar to that of humans. The capacity to produce IFN reduces with age. It was found that genes of virtually all studied cytokines are expressed in blood cells and hence, in immune system cells.

Key Words: *cytokines; interferon-α; interferon-γ; interferon status*

Such human diseases as dysentery, measles, smallpox, poliomyelitis, rubella, AIDS, *etc.*, are simulated only in laboratory primates. Correct evaluation of the results of these model studies on monkeys necessitates adequate evaluation of the physiological systems of experimental animals. Published data on the status of the cytokine system (IFN, IL, and other lymphokines) of normal monkeys are incomplete, and there are no summary data on the functioning of these systems in the entire immune system. On the other hand, studies of the role of cytokines, playing the key regulatory role in the maintenance of homeostasis, mediating the majority of immunological reactions [5,7], in a model system on monkeys will help to better understand the essence of immunopathological conditions in humans.

We studied the functioning of the cytokine system in primates.

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MATERIALS AND METHODS

The study was carried out on 114 monkeys. The IFN status was studied in 80 monkeys of different species: 31 *M. mulatta*, 24 *P. hamadryas*, and 25 *C. aethiops*. Age-specific production of IFN was studied in 9 monkeys: 5 *M. mulatta* and 4 *M. iris*.

The age of experimental monkeys varied from 1 to 25 years. The main group included young animals (1-2 years), adult (3-17 years), and old animals (17 years and older).

Isolation and titration of IFN was carried out as described previously [1,3]. The IFN status was regarded as the capacity of monkey lymphocytes to respond by an *in vitro* interferon reaction to an adequate stimulus. Evaluation of the IFN status included measurements of IFN-α and IFN-γ, spontaneous IFN production and its serum (circulating) level. Newcastle disease virus (10^9-10^{11} cytopathic doses; 50.0/0.1 ml) served as IFN-α inductor, Con A (T-cell mitogen; 20 μg/ml, PanEco) served as IFN-γ inductor.

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Interferon preparations were titered on target SPEV (porcine embryo kidney) cells against 100 cytopathic doses of murine encephalomyocarditis virus, titered on SPEV culture cells. The method for isolation and titration of IFN was described in detail previously [2,4,6].

The value inversely proportional to the maximum dilution of the preparation, at which the specific destruction of target cell monolayer reduced by 50%, was taken for IFN titer.

The production of other cytokines was evaluated on 25 clinically healthy *M. mulatta* with the known IFN status.

Activities of 11 cytokines mRNA in monkey peripheral blood mononuclear cells were studied by reverse transcription and PCR. Isolation of RNA was carried out by acid guanidine thiocyanate phenol-chloroform extraction. Primer pairs for the following cytokines were used: IFN-α, IL-6, IL-8, IL-1β, IL-2, IL-4, IL-10, TNF-α, IFN-γ, IL-18, and IL-12. β-Actin served as the positive control. The PCR results were recorded electrophoretically in 2.5% agarose gel stained by ethidium bromide. The nucleotide sequences were identified by G 1758 marker for electrophoresis (Promega) [3,5-7].

RESULTS

The IFN status of *M. mulatta* was evaluated in 31 clinically healthy animals of both sexes aged 3-8 years. The highest production of IFN-α and IFN-γ was detected in 4 animals aged 4 years (256-512 and 128 U/ml, respectively). Lymphocyte capacity to produce IFN did not depend on animal sex (Table 1).

The IFN status was studied in 24 clinically healthy *P. hamadryas* aged 1-14 years (Table 1). The highest production of IFN-α (256 U/ml) and IFN-γ (128 U/ml) was detected in 5 monkeys aged 3.6-8.0 years. Monkeys aged 9-14 years produced IFN in lower titers (32-64 U/ml; Table 1).

In *C. aethiops* the highest capacity to IFN production (128-256 U/ml) was observed in animals of both sexes aged under 3 years, while the lowest production was recorded in animals aged 5-7 years (Table 1).

Hence, studies of the IFN status in monkeys of different species detected their different capacity to IFN production. The highest activity of IFN system functioning was observed in *M. mulatta*, lower activities in *C. aethiops* and in *P. hamadryas*.

Depression of IFN system functioning in elderly humans [3,4] suggested evaluation of the IFN status

TABLE 1. The IFN Status of Clinically Healthy Laboratory Primates (*M*±*m*)

Monkey species	Number	Age, years	Sex	IFN, U/ml			
				α	γ	spontaneous	serum
M. mulatta (*n*=31)	13	5-8	males	64-128	32-64	4	4-8
	10	5-8	females	128	32-64	4-8	4
	4	3-3.5	males	128	64	4	4
	4	4	males	256-512	128	4-16	4-16
Mean values of IFN status				146.58±19.27	60.80±5.11	5.42±0.57	5.55±0.57
P. hamadryas (*n*=24)	9	1	females	128	64	4	4
	5	3.6-8	females	256	128	4	4
	10	9-14	males	32-64	16-64	<4-8	<4
Mean values of IFN status				120±18	48.6±3.6	4	4
C. aethiops (*n*=25)	7	1-3	males	128-256	64	4	4
	8	4-6	males	64-128	32-64	4	4
	5	2-3	females	128-256	64	4	4
	5	5-7	females	64	64-128	4	4
Mean values of IFN status				128.0±14.3	64±7	4	4

TABLE 2. The IFN Status of Old Animals (*M*±*m*; *n*=9)

Species	Number	Age, years	IFN, U/ml			
			α	γ	spontaneous	serum
M. mulatta	5	21-22	32	16-32	4	4-8
M. iris	4	24-25	32-64	16-32	8	4
Mean values of IFN status			39.11±4.70	23.11±2.80	5.77±0.70	5.77±0.70

TABLE 3. Cytokine Production in Normal Monkeys (*n*=25) and Healthy Volunteers (*n*=100)

Cytokine mRNA	Normal monkeys with cytokine mRNA, %	Normal subjects with cytokine mRNA, %
IFN-α	96	5
IFN-γ	40	30
TNF-α	56	75
IL-1β	64	35
IL-2	76	5
IL-4	28	5
IL-6	80	20
IL-8	20	10
IL-10	36	20
IL-12	36	70
IL-18	84	30

of old monkeys. Similarly as in humans, the production of IFN-α and IFN-γ was significantly reduced (Table 2).

In addition to IFN system values, the cytokine production was studied in clinically healthy monkeys. These parameters were measured in 25 *M. mulatta*. The status of the IFN system and of other cytokines in health was evaluated by the cytokine genes expression (by the production of their mRNA). The data on the cytokine mRNA in normal monkeys and humans are summed up in Table 3.

The IFN-α, IL-2, and IL-4 mRNA was virtually not detected in healthy volunteers; TNF-α and IL-12 mRNA was detected in 70% examinees; and mRNA of other cytokines was detected in 10-30% normal subjects. In the monkeys IFN-α, IL-2, IL-6, IL-18 mRNA was detected in 80-100% animals, IL-1β and TNF-α mRNA in 60%, and IFN-γ, IL-4, IL-8, IL-10, and IL-12 mRNA was detected in 20-40% monkeys.

Hence, lymphocytes of monkeys of different species react to adequate stimulation by IFN production. The production of IFN is the maximum in animals aged 4-8 years irrespective of species and sex. With aging lymphocyte capacity to react to IFN-α inductors reduces 3.8 times and to IFN-γ inductors 2.6 times, which is in line with the values in humans [3,4]. On the whole, the IFN status of monkeys is similar to that of humans [6], which suggests monkeys as an adequate model for reproduction of human immunopathological conditions.

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