Ir-GENE-CONTROLLED T CELL PROLIFERATION IN RESPONSE TO Igk-1^b IMMUNOGLOBULIN ALLOTYPE IN RATS

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The Igk-1 locus controls the formation of two allelic forms of light chains of rat IgG of the k type (Igk-1^a and Igk-1^b), which differ with respect to 11 amino-acid residues in their C_L domains. Inbred lines of rats have one or other allelic variant of the k-chains in the composition of 95% of their molecules of both serum and membrane Ig [6]. The writers showed previously that the ability of inbred rats to form anti-Igk-1^b-antibodies and to generate T helper cells and T cells of delayed-type hypersensitivity (DHT) *in vivo* is under the control of the gene of the Ir-Igk-1^b immune reaction, linked with the main histocompatibility complex (MHC, RT-1 of rats). WAG rats (RT-1^u haplotype) have been found unable to develop immune reactions to Igk-1^b antigen [1, 2].

The writers have developed a system of antigen-specific proliferation of rat T lymphocytes in vitro in response to the Igk-1^b haplotype and have investigated Ir-control and MHC-restriction of allotype-specific T cells.

METHODS

Female August rats (RT-1^c, Igk-1^a), and female WAG rats (RT-1^u, Igk-1^a) from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, Fisher rats (RT-1^b, Igk-1^b) bred at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and also (WAG August)F_1 hybrid rats and MSU/b (Igk-1^b) rats, bred and maintained in the writers' laboratory, were used. The age of the animals was 8-12 weeks and their weight 120-140 g.

IgG_2 of MSU/b and Fisher rats (Igk-1^b) and also of August rats (Igk-1^a), isolated by sulfate fractionation of sera followed by chromatography on DEAE-cellulose (DE-32, from Whatman, England) were used as allotypic reagents. The Fab fragment (Igk-1^b) was obtained by papain proteolysis of Fisher IgG_2 and isolated by chromatography on DE-32 [1]. Hen Ig (HGG) was obtained by sulfate fractionation of hen serum. Other antigens (AG) were obtained as follows: ovalbumin (OA) from Serva, West Germany, and purified tuberculin (PPD), from the Seruminstitut, Denmark.

Rats were immunized by subcutaneous injection (into the hind foot pads) of 0.1 ml of a mixture of AG solution and Freund's complete adjuvant (FCA), containing *Mycobacterium tuberculosis* H37Ra (from Difco, USA). The dose of AG per animal was: Igk-1^b 250 μg, OA 150 μg, and HGG 150 μg. A suspension of popliteal lymph node cells was obtained and washed in Eagle's medium containing 5% inactivated bovine serum (BS) and 100 U/ml each of penicillin and streptomycin (P/S).

Immune T lymphocytes were isolated by fractionation on columns with nylon wadding (Penwall) [4] or on polystyrene Petri dishes (Linbro, England), covered with rabbit antibodies to the $F(ab)_2$-fragment of rat Ig [5]. The T lymphocytes were transferred into complete RPMI-1640 culture medium containing 3 mM of L-glutamine, 20 mM of HEPES buffer (all from Flow laboratories, England), P/S, $5 \cdot 10^{-5}$M 2-mercaptoethanol (Sigma, USA), and 5% inactivated human group AB serum.

T cells numbering $4 \cdot 10^5$ in 0.1 ml of complete medium and an equal volume of the dilutions of AG were introduced into wells of a flat-bottomed 96-well plate (3040, Falcon, USA). The cells were cultured for 96 h at 37°C in an atmosphere with 5% CO_2. ^{3}H-thymidine (specific

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TABLE 1. Antigen-Specific Proliferation of T Lymphocytes of August, WAG, and (WAG × August)F_1 Rats)

Primary immunization	Secondary immunization	Line of rats		
		August	WAG	(WAG×August) F_1
Freund's incomplete adjuvant	HGG	130±37 (1,35; 1,5)	—	—
	PPD	286±69 (1,8; 1,75)	—	—
	IgG_2 (Igk-1^b)	127-56 (0,9; 1,15)	—	—
	con A	89 300±1 500 127; 92; 210)	—	—
	Control	150±70	—	—
IgG_2 Igk-1^b in FCA H37Ra	IgG_2 Igk-1^b	11 080±260 (4,3; 5; 4; 3,8)	1 580±436 (1,1; 0,9; 1,6; 1,21)	2 959±420 (4,25; 2,59; 4,7; 4,3)
	Fab Igk-1^b	7 940±510 (3,25; 2,8)	—	—
	IgG_2 Igk-1^a	3,925±1 420 (1,51; 1,6; 1)	—	—
	PPD	55 905±1 474 (17;125; 23)	12 750±2 107 (13; 26; 16)	22 560±5 786 (14; 11; 16)
	HGG	3 873±613 (1,4; 1,85)	—	—
	Control	2 600±519	850±354	680±415
HGG in FCA	HGG	45 514±3 560 (20,7; 24,3)	34 020±318 (20,9; 19,6)	10 143±1 000 (10; 14,3)
	IgG_2 (Igk-1^b)	2 146±786 (0,97; 1,1)	3 445±414 (1,57; 1,85)	—
	OA	3 617±165 (1,6; 1,9)	—	—
	Control	2 200±803	1 770±1 424	1 100±51
OA in FCA	OA	5 533±210 (17,1; 21)	28 005±12 019 (37, 23,7)	—
	Control	274±120	750±156	—

Legend. Results of 1 experiment (in cpm) are shown. Results of similar experiments in SI for each experimental group given in parentheses; con A) concanavalin A.

radioactivity 2 Ci/mmole) in 20 µl of medium was added to each well 16-18 h before the end of culture. Labeled cells were collected on fiberglass filters on a cell harvester, and the incorporated radioactivity was determined on a "Tricarb" scintillation counter. The cultures were set up in 3 parallel experiments and the results were expressed in cpm or stimulation indices (SI).

$$SI = \frac{\text{cpm in experiment}}{\text{cpm in control}}.$$

To obtain a suspension of AG-presenting cells (APC) 10 ml of a 3% solution of peptone was injected intraperitoneally into nonimmune rats 48 h before collection of the peritoneal macrophages. The cells were obtained by flushing out the peritoneal cavity of the rats with Eagle's medium with 5% BS, P/S, and heparin (15 U/ml; from Reanal, Hungary), and after washing they were incubated at the rate of 10^7 cells to 1 ml RPMI-1640 medium, 3 mM L-glutamine, and P/S for 1.5 h at 37°C with AG in final concentrations of Igk-1^b and Igk-1^a of 300 µg/ml, PPD 150 µg/ml. To inhibit incorporation of ^{3}H-thymidine mitomycin C (Sigma) was added to the suspension of APC up to a final concentration of 40 µg/ml, and the mixture was incubated for a further 30 min at 37°C. After 4 or 5 washings the APC were transferred into complete medium and $5 \cdot 10^4$ to 10^5 cells were added to each well of a 96-well plate. Further culture was carried out as described above.

RESULTS

The secondary response of the rats' T lymphocytes to Igk-1^b, just as to other AG (PPD, OA, HGG), was recorded 7-28 days after primary immunization of the animals. The kinetics of the response of T lymphocytes of August rats to Igk-1^b is shown in Fig. 1. The proliferative response rose to a peak 96 h after the beginning of culture, and its course coincided with the kinetics of the response to another AG, namely PPD. The intensity of proliferation of

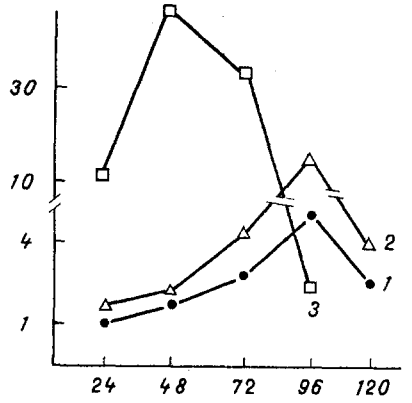

Fig. 1. Kinetics of antigen-specific proliferation of T lymphocytes of August rats *in vitro*. Abscissa, duration of culture (in h); ordinate, SI. 1) response of T lymphocytes to 200 μg/ml of Igk-1^b; 2) to 100 μg/ml of PPD; 3) to 5 μg/ml of con A.

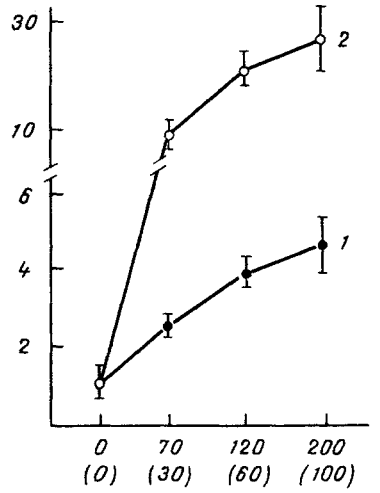

Fig. 2. Antigen-specific proliferation of T lymphocytes of August rats *in vitro*. Abscissa, concentration of Igk-1^b (in μg/ml), PPD in parentheses, ordinate, SI. 1) response to Igk-1^b; 2) response to PPD. Results of 2 experiments (M ± m).

the T lymphocytes depended on the Igk-1^b concentration in the culture medium and its dose-dependence was similar to that observed in responses to PPD (Fig. 2), HGG, or OA. Thus the basic parameters of AG-dependent proliferation of T lymphocytes to Igk-1^b were similar to those of responses to the foreign antigens used.

The results given in Table 1 illustrate the strict immunologic specificity of the response of T lymphocytes to Igk-1^b and other AG. A response *in vitro* developed only to stimulation by the AG used for primary immunization. Nonimmune T lymphocytes did not proliferate in response to any of the protein AG tested. Further evidence of the specificity of the response to Igk-1^b (Table 1) was given by proliferation of T lymphocytes of August rats to the

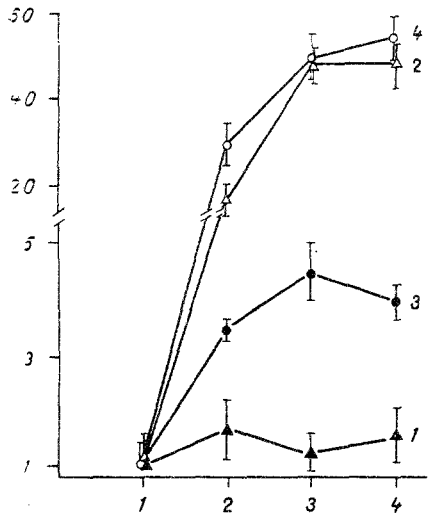

Fig. 3. Presentation of APC of August and WAG rats to (WAG × August)F_1 Igk-1^b T lymphocytes. Abscissa, dilution of Igk-1^b-pulsed or PPD-pulsed APC by Igk-1^b-pulsed control APC (C-APC); 1) $8 \cdot 10^4$ C-APC; 2) $6.4 \cdot 10^4$ C-APC + $1.6 \cdot 10^4$ Igk-1^b-(PPD-)pulsed APC; 3) $4 \cdot 10^4$ C-APC + $4 \cdot 10^4$ Igk-1^b-(PPD-)pulsed APC; 4) $8 \cdot 10^4$ Igk-1^b-(PPD-)pulsed APC; ordinate, SI: 1) response to Igk-1^b-pulsed APC of WAG rats; 2) to PPD-pulsed APC of WAG rats; 3) to Igk-1^b-pulsed APC of August rats; 4) to PPD-pulsed APC of August rats. Cultures set up in 4 parallel experiments (M ± m). Results of 1 of 3 identical experiments shown.

Fab-fragment of the Igk-1^b molecules and the absence of proliferation of anti-Igk-1^b T cells in response to August IgG_2, which carries the alternative immunizing allotype (Igk-1^a).

These results show that Igk-1^b-dependent proliferation is not connected with any possible activation of T cells through interaction of their Fc-receptors with the Fc-site of the IgG_2 molecules, and also with the recognition of denaturation determinants which could arise in the process of isolation of IgG_2 (Igk-1^b), by T lymphocytes. As Table 1 shows, the responses to Igk-1^b was characterized by lower stimulation indices (SI 3.8-5) than the response to foreign antigens (SI 10-37). This is evidently due to the low frequency of clones of antiallotypic T lymphocytes detected in the AG-specific proliferation test, and also by the nature of the Igk-1^b (this molecule carries only two serologically identifiable antigenic determinants). Similar levels of T-cell proliferation were obtained in an investigation of responses of mice to hapten-modified syngeneic and allogeneic Ig [3].

Another important result was obtained on comparative analysis of the response of T cells from August, WAG, and (WAG × August)F_1 rats to Igk-1^b (Table 1). The experiments showed that T lymphocytes of August and F_1 rats could respond to Igk-1^b (SI 3.8-5), whereas T lymphocytes of immunized WAG rats gave no antiallotypic response (SI 0.9-1.8). These results agree fully with those of a previous analysis of the ability of rats of these lines to form antiallotypic antibodies and to generate T helper cells and T cells of delayed-type hypersensitivity in vivo [1, 2]. Thus the proliferative response of the T cells, tested *in vitro*, also was under the control of the Ir-Igk-1^b gene.

Further confirmation of this conclusion was obtained in a reconstructive system of the proliferative response, using Igk-1^b AG-pulsed APC to stimulate immune T lymphocytes. In these experiments T lymphocytes of (WAG × August)F_1 rats, immunized with Igk-1^b, were stimulated

by Igk-1^b-pulsed APC of the responding line of August rats and the nonresponding WAG line. PPD-puled APC of these lines were used as the positive control.

The results of these experiments showed (Fig. 3) that only presentation of Igk-1^b by cells of August, but not of WAG, rats induced specific stimulation of immune (WAG × August)F_1 T lymphocytes. August and WAG APC were equally effective under these circumstances in presentation by the T cells of another AG, namely PPD, the response to which was comparable in rats of these two lines (Table 1).

The results thus demonstrate the MHC-dependent Ir gene-control of the stage ofmacrophage–T cell interaction in the course of recognition of the Igk-1^b allotype by T lymphocytes. They also show that the Igk-1^b alloantigen is recognized by T cells in association with characteristic MHC products. Absence of immunoreactivity to Igk-1^b in rats may accordingly be due to deficiency of clones of RT-1^u-restricted (WAG) T lymphocytes specific for Igk-1^b, and to ability to form functional complexes of Igk-1^b and RT-1^u products at the APC level (AG-specific presentation defect) or to generation of RT-1^u-restricted Igk-1^b-specific T suppressors (WAG).

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EFFECT OF VITAMIN E ON OXIDATIVE METABOLISM OF MACROPHAGES

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Adaptation of polymorphonuclear leukocytes (PNL), macrophages, and lymphocytes is accompanied by a respiratory "burst," characterized by intensification of oxidative metabolism and release of active oxygen radicals [3, 10]. The latter are largely responsible for the bactericidal properties of phagocytes but they may be toxic for surrounding tissues and for the cells themselves [7, 9, 13]. The important role of the enzyme superoxide dismutase (SOD), a modulator of oxidative metabolism of PNL which possesses anti-inflammatory properties, was demonstrated previously [9]. Free-radical reactions are inhibited by antioxidants and, in particular, by vitamin E, which blocks H_2O_2 production by PNL [4]. However, the mechanism of this phenomenon is largely unexplained.

The aim of this investigation was to study oxidative metabolism of macrophages under conditions of vitamin E deficiency, characterized by activation of lipid peroxidation (LPO) *in vivo*.

METHODS

Experiments were carried out on growing male Aug-Lac rats weighing initially 40–60 g and kept for 2 months on a semisynthetic balanced diet of the following composition (in %): protein 24.5 (casein 21, wheat gluten 3.5), carbohydrate 59.4 (starch 53.5, sucrose 5.9), fat 10

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