IMMUNIZATION OF HUMAN LYMPHOCYTES WITH EPSTEIN-BARR VIRUS CAPSID ANTIGENS IN VITRO

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Human monoclonal antibodies (HMAB) are a powerful and promising therapeutic agent for the very near future. Advantages of HMAB over murine monoclonal antibodies are familiar [13, 14], but several factors have delayed their use. The first of these is the impossibility of immunizing a human being with each and every antigen, and for that reason efforts to obtain HMAB have been limited to the use of donors with persistent infections or of volunteers [15]. However, the creation of specific conditions for fully effective antigenic stimulation of B lymphocytes in culture will perhaps enable this difficulty to be overcome [10, 14]. The most accessible material for this procedure consists of peripheral blood lymphocytes (PBL), although it has been found that immunization with PBL in vitro is ineffective because of the active function of suppressor T cells, and it is necessary to use special agents capable of abolishing the action of immunosuppression [2, 3, 10].

The conditions of immunization of human lymphocytes in vitro (from blood and tonsils) with viral capsid antigens (VCA) of Epstein-Barr virus (EBV), associated with a number of human pathologies [8], were studied.

EXPERIMENTAL METHOD

PBL, tonsillar lymphocytes (TL) from healthy blood donors, and neonatal umbilical vein lymphocytes (UL) were used. Mononuclears were obtained by the method in [4]. Antibody titers in the donors' serum were determined by the immunofluorescence method on films of V95-8 marmoset cells, stimulated by phorbol myristate acetate (PMA) [11]. To determine antibodies (AB) of the G class in serum and AB of the M and G classes in culture fluid (CF), we used enzyme immunoassay (EIA). A purified preparation of EBV, strain B95-8 [12] or PMA-stimulated B95-8 cells, immobilized on EIA planchets, were used as AG. The virus was adsorbed in a concentration of 5 μg/ml (as protein) in 0.1 M borate buffer, pH 9.0, at 4°C. The wells were blocked with PBS containing 0.05% Tween-20 and 0.5% BSA. The B95-8 cells were stimulated by PMA and immobilized as described in [7]. Double dilutions of serum or CF were incubated in planchets with AG for 18 h at 4°C, washed off with the blocking buffer, and treated with antispecific conjugate with peroxidase for 2 h at 20°C. The substrate was *o*-phenylenediamine, 0.2 mg/ml, in 50 mM phosphate buffer, pH 5.6, containing 0.005% H_2O_2. Optical density was measured at 495 nm on a Multiscan photometer ("Titertek," England). PBL from a healthy blood donor was used as the cellular antigen control.

For immunization in vitro fresh samples of PBL, TL, and UL were cultured at 37°C, in a density of 10^6 cells/ml, for 1-9 days in 75-cm^2 plastic flasks ("Costar," USA). We used Iskov's medium with 40 μg/ml of gentamicin, 5×10^5 M 2-mercaptoethanol, and 5% fetal serum ("Myoclone," from "Gibco," USA), with or without the addition of: pokeweed mitogen (PWM) 5 μg/ml, 500 ng/ml of EBV, strain P3HR1, and 7% by volume of CF with B-cell growth factor (BCGF) of the Namalwa line [1]. The concentration of anti-VCA antibodies of class M and G and also the number of viable cells in 1 ml of CF, based on nonuptake of trypan blue, were determined daily in aliquots of CF.

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TABLE 1. Results of Determination of Anti-VCA-Antibodies in Culture Fluid of Lymphocytes during Immunization in Vitro

Type of cells	Donor	Titer of anti-VCA-antibodies in serum	Stimulators			Results of determination of anti-VCA-antibodies, A (495 nm)		
			PWM	P3HR1 virus	Namalwa BCGF	AG:EBV, strain B95-8, IgM/IgG	AG:PMA stimulated B95-8 cells, IgM/IgG	control AG:PBL, IgG
	LOS	1200	–	–	–	0,18/0,22	0,24/0,28	0,28
			–	–	+	0,15/0,24	0,18/0,33	–
			–	+	+	0,54/0,96	1,13/1,22	0,36
			+	+	+	1,15/1,24	1,88/1,62	0,29
			–	–	–	0,09/0,15	–	–
PBL	MOS	1600	–	+	+	0,36/1,12	0,87/1,52	0,22
			+	+	+	0,84/1,67	1,13/1,86	0,39
	ASD	1800	–	–	–	0,10/0,09	0,32/0,56	–
			–	+	+	0,44/0,81	0,60/1,14	0,12
			+	+	+	0,12/1,53	0,72/1,37	0,27
TL	AAE	2200	–	–	–	0,12/–	–	–
			–	–	+	0,15/0,24	–	–
			–	+	+	0,72/1,87	–/1,24	0,35
	AAG	1400	–	–	–	0,07/0,19	0,26/0,19	0,07
			–	–	+	–	0,13/0,54	0,38
			–	+	+	1,13/1,75	1,04/1,52	–
			+	+	+	1,18/1,90	1,77/1,95	0,32
PBL	NAS*	120	–	–	–	0,06/0,13	0,11/0,14	–
			–	+	+	0,17/0,09	0,22/0,18	0,08
			+	+	+	0,38/0,46	0,82/0,14	0,15
			–	+	–	0,22/0,17	–	–
	NV1*	50	–	–	–	0,08/0,14	0,10/0,17	0,06
			–	+	+	0,44/0,12	0,66/0,18	0,08
			+	+	+	0,58/0,29	0,78/–	–
UL	NV2*	<10	–	–	–	0,10/0,19	–	–
			–	+	+	0,36/0,21	–	–
			+	+	+	0,77/0,44	0,88/–	0,12
	NV3*	<10	–	–	–	0,08/0,16	–	0,10
			–	+	+	0,11/0,17	–	0,12
			+	+	+	0,12/0,34	0,42/0,15	

EXPERIMENTAL RESULTS

Successful immunization in vitro requires an optimal combination of AG, growth factors, nonspecific serum factors, and type of lymphoid cells [10]. We therefore used VCA AG of untransformed strain P3HR1 of EBV [12], BCGF from CF of Namalwa cells [1], and additional stimulation of lymphocytes by PWM.

Lymphocytes of donors LOS, MOS, ASD, AAE, AAT, NAS NV1, NV2, AND NV3 were stimulated with PWM, AG of virus P3HR1, and Namalwa BCGF in order to generate an immune response in vitro. Table 1 gives the results of experiments for EBV from seropositive and seronegative donors. Clearly, during culture of lymphocytes of any of the donors in ordinary medium spontaneous secretion of antibodies to EBV was not observed; only stimulation by BCGF led to insignificant cell proliferation, but not to specific secretion. Simultaneous addition of AG and BCGF to the medium induced considerable secretion of specific anti-VCA-antibodies of the M and G classes, i.e., generation of a secondary immune response, whose intensity correlated positively with the titer of anti-VCA-antibodies in the donor's serum. When tonsillar lymphocytes were used, more intensive immunization was observed, evidently due to the larger number of stimulated B-cells in the tonsils. Maximal stimulation was observed on the addition of PWM also to the system: the number of AB-secreting cells was increased by 1.5-2 times. In some cases we observed synergism of action of PWM and EBV AG. A similar situation also has been described [9] in the case of immunization of human PBL with AG of type I herpesvirus, serologically closely similar to EBV.

The attempt to generate the primary response in cultures of EBV-negative donors NAS and NV1, 2, and 3 was successful only in the case of combined addition of PWM, viral AG, and BCGF to the medium; under these circumstances secretion mainly of class M AB was observed, i.e., the response evidently developed by a primary mechanism. Of the three completely negative donors (UL), successful immunization in vitro occurred only in one case (donor HV2), and only if PWM was added to the medium. It will be evident that in this case also, synergism was manifested between the polyclonal stimulation by the mitogen and the known property of EBV to induce polyclonal IG synthesis also in lymphocyte cultures [9]. Similar results of analysis of secreted AB with use of two different types of EBV AG are evidence of the specific antiviral character of the immune response.

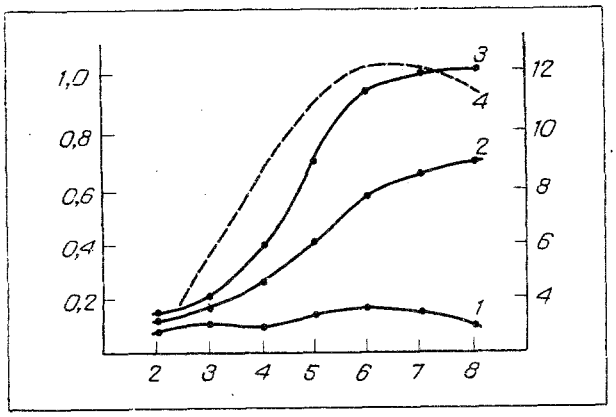

Fig. 1. Kinetics of immunization in vitro of lymphocytes from donor NAS, with low reactivity, by EBV antigens in the presence of PWM and Namalwa BCGF. Abscissa, culture time (in days), ordinate, left — light absorption at 495 nm (in optical density units); ordinate, right — number of viable cells, × 10^5/ml. Continuous lines represent anti-VCA-activity of immunoglobulins: 1) class G in absence of AG, BCGF, and PWM; 2) class G in response to stimulation by AG, BCGF, and PWM; 3) class M in response to stimulation by AG, BCGF, and PWM. Broken line 4 represents proliferation of lymphocytes in response to stimulation by AG, BCGF, and PWM.

During the study of the kinetics of the primary humoral AG-response in a culture of NAS cells (Fig. 1) it was found that optimal secretion of specific AB was observed toward the 7th day of culture, which is virtually the same as the optimum of proliferation of these cells. It is important to note that in an analogous system, AG-specific lymphoblasts for subsequent hybridoma formation can be isolated [5]. During the next 3-5 days of culture, cell proliferation diminished sharply, and the cultures lost their viability and secretory activity.

The system of immunization in vitro described above does not rule out operations to remove suppressor cells, and the efficacy of generation of the primary immune response in vitro, which we succeeded in obtaining, is therefore surprising. This fact is probably linked with the ability of EBV to induce polyclonal AB synthesis and proliferation of B cells [8], more especially because the kinetics of the process resembles that observed under the influence of PWM. Considering that EBV can bind with B cells not only as an AG, but also as a specific B-lymphotropic virus (through CR2-receptors) [6], such a combination may determine the unique ability of EBV to generate an immune response in PBL culture. It would be convenient to use such a property in the future in order to create conjugates of any T-dependent antigen with EBV VCA proteins with the aim of immunizing PBL.

It follows from the results given above that: 1) a primary immune response can be generated in cultures of lymphocytes from EBV-negative donors in the presence of three components: PWM, low concentrations of EBV AG, and partially purified BCGF; 2) there are evidently distinct advantages from the use of tonsillar lymphocytes for this purpose.

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