the same carrier specificity as delayed hypersensitivity (Oppenheim *et al.*, 1967; Senyk *et al.*, 1971).

In-vitro lymphocyte stimulation, which is thought to represent mainly cell-mediated immunity against HBAG, was shown up to 72 weeks after the last signs of hepatitis in nine of our group of 12 patients who had recovered from the disease. Only five showed transient antibodies, and in these five an immune response against HBAG could be shown with in-vitro lymphocyte stimulation after the HB antibodies had disappeared. Two of the three patients who showed no stimulation were tested within four weeks of the disappearance of HBAG from their serum, but in that interval a cell-mediated immune response may not be detectable with in-vitro lymphocyte stimulation.

The fact that lymphocyte sensitization as well as antibody formation were also found in the group of laboratory technicians shows that a complete immune response can develop after exposures to HBAG which do not result in an attack of hepatitis. In two cases antibodies were detected without in-vitro lymphocyte stimulation. Perhaps such differences in the immune response may be related to the dose of HBAG, the subtype, the mode of transmission, or to genetic factors.

Most proteins lose their ability to induce antibody formation after heat-denaturation but remain as effective as untreated proteins in inducing delayed hypersensitivity (Gell and Benacerraf, 1959). Purified HBAG denatured by reduction and alkylation was found to have lost its ability to induce a humoral reaction in guinea-pigs but its ability to induce delayed hypersensitivity was unaffected (Vyas *et al.*, 1972). In contrast, we found that heat-inactivated purified HBAG elicited an antibody response in guinea-pigs. Moreover, it was found to be immunologically identical to untreated HBAG in immunodiffusion using monospecific antisera. Krugman and Giles (1973) found

antibodies against HBAG in some people after immunization with heat-inactivated HBAG-rich serum, while Soulier *et al.* (1972) found antibodies in all of seven persons immunized with a higher dose. In our studies purified, heat-inactivated HBAG stimulated sensitized lymphocytes in vitro.

These findings suggest that vaccination with purified, heat-inactivated HBAG might induce not only humoral but also cell-mediated immunity against serum hepatitis. Further studies are needed to show whether cell-mediated rather than humoral immunity against HBAG offers protection against hepatitis B.

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Changes in Antigenic Nature of Lymphocytes Caused by Common Viruses

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Summary

Healthy human lymphocytes were incubated in the presence of influenza A2/Singapore/57, herpes simplex type 1, or adenovirus type 2. After two days the cultures were inactivated by irradiation. Fresh lymphocytes taken from the same donor were then found to react to the virus-treated cells in short-term cultures. We suggest that this reactivity is due to a change in the surface characteristics of the lymphocytes brought about by the presence of the virus. This may account for anomalous reactions in mixed lymphocyte cultures, and a similar effect in vivo might cause accelerated graft rejection.

Introduction

Recently we reported unexpected reactivity between the lymphocytes of two identical twins (Pegrum *et al.*, 1972). At the time it was noticed that the twin whose cells caused the reactivity was suffering from a heavy cold. When he recovered no reaction occurred between these siblings' lymphocytes. This observation suggested the possibility that the surface antigens on the stimulating lymphocytes had been altered by the viral infection. Such a change might account for the observation that lymphoblastoid cell lines obtained from patients with glandular fever cause reactivity of the subject's own lymphocytes after recovery (Steel and Hardy, 1970).

To test this hypothesis further lymphocytes were cultured in a nutrient medium with three viruses. After incubation cultures were irradiated and the cells washed. These were then placed in contact with fresh autologous lymphocytes and the reactivity was assessed.

Materials and Methods

PREPARATION OF VIRUSES

Influenza A2/Singapore/57 virus was grown at 37°C in the allantoic fluid of 10-day-old fertile hens' eggs. After three days

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the allantoic fluid was removed and the virus recovered in the supernatant after centrifugation. Herpes simplex type 1 and adenovirus type 2 were grown in human diploid embryonic fibroblasts (M.R.C.5) in Eagle's medium with 10% fetal calf serum and 10% tryptose phosphate broth. After three days the cell cultures were repeatedly frozen and thawed to break up the cells and release the virus. The cell fragments were removed by centrifugation and the supernatant was used as the source of virus. The influenza virus was titrated against O-positive blood cells and the same concentration used in all experiments. The titre of the herpesvirus and adenovirus was unknown but aliquots of the same batch were used throughout.

PREPARATION OF CULTURES

A 30-ml sample of blood from healthy donors was taken into heparin; the leucocyte-rich plasma was allowed to separate and was then removed. Lymphocytes were separated from the plasma using a column of nylon fibre. The column was prepared by packing a 10-ml syringe with nylon fibre and then washing through with Eagle's M.E.M. Leucocyte-rich plasma was introduced through the top and allowed to soak into the nylon. The column was incubated at 37°C for 30 minutes and lymphocytes were obtained by washing with 20 ml of Eagle's medium. This method gives over 95% of lymphocytes.

A cell count was performed and the volume adjusted to give approximately 2×10^6 cells/ml in Eagle's M.E.M. (+ Hepes) and 10% fetal bovine serum. Aliquots of this cell suspension were incubated for 48 hours at 37°C with (a) influenza A2/Singapore/57, (b) herpes simplex type 1, and (c) adenovirus type 2. Further aliquots were incubated for the same period with virus which had been inactivated by irradiation (5,000 rads). A control sample of lymphocytes alone was set up at the same time. After two days all cultures were exposed to 5,000 rads to inactivate both virus and lymphocytes. The cells were subsequently washed three times with Eagle's M.E.M. to remove any free virus. Before irradiation an aliquot was removed from each culture and pulsed with ^3H -thymidine as described below to assess any reactivity at this stage.

A further 30 ml of blood was taken from the same donor and another lymphocyte preparation made as described. Duplicate microcultures were then prepared by placing equal numbers of the irradiated cells with fresh, untreated lymphocytes to give a total volume of 1 ml with 1×10^6 cells. Control cultures of lymphocytes and irradiated cells alone were prepared. Further controls of lymphocytes with the supernatant of washed irradiated cells were also prepared. The cultures were incubated for five days at 37°C. Sixteen hours before the end of culture 2.5 μCi ^3H -methylthymidine (with a specific activity of 5.0 Ci/mmol) was added to each tube.

Samples were prepared for scintillation counting by placing duplicate 0.1-ml aliquots from each culture on Whatman paper discs (grade 3 mm, size 1.9 cm). These discs were dried, treated with 20% trichloroacetic acid for 10 minutes at 4°C, and then washed with 3% perchloric acid. Further washing and drying was effected with methanol and ether. The dried discs were placed in 20 ml scintillation fluid (4 g PPO and 100 mg dimethyl POPOP/l. analar toluene) and the radioactivity was assessed using a Nuclear Enterprise β counter.

Results

The cells treated with viable virus after 48 hours and before irradiation showed no increase in DNA synthesis. The irradiated virus-treated lymphocytes placed with fresh, untreated lymphocytes from the same donor for five days caused an increase in DNA synthesis when compared with identical lymphocytes cultured with irradiated lymphocytes without viral pretreatment (see table). The results were not uniform in the six donors tested, and in donor 5 a negative effect was produced by the cells pretreated with influenza virus. But in all other instances an effect was produced, and the degree of response was consistent in each individual. The range and mean results of three separate cultures using donor 1 are shown in the chart.

Though the dose of irradiation used to inactivate the lymphocytes probably does not kill the viruses, DNA synthesis of newly forming virus particles cannot be responsible for the uptake of thymidine. Viral DNA synthesis would be expected to occur

Uptake of ^3H -Thymidine by Lymphocytes in Mixed Culture with Autologous Irradiated Pretreated Lymphocytes. Results expressed as c.p.m./ 10^6 Cells

Donor	Lymphocytes + Untreated Irradiated Lymphocytes		Lymphocytes + Irradiated Flu-treated Lymphocytes		Lymphocytes + Irradiated Herpes-treated Lymphocytes		Lymphocytes + Irradiated Adeno-treated Lymphocytes	
	Individual Values	Average Values	Individual Values	Average Values	Individual Values	Average Values	Individual Values	Average Values
1	87	93	286	380	2,615	2,244	351	368
	91		535		3,152		457	
	94		225		1,336		279	
	99	138	474	990	1,873	866	385	697
	146		995		1,017		749	
	148		1,063		742		619	
	133	422	1,003	812	748	1,579	721	532
	127		900		960		699	
	271		936		1,809		496	
	628	266	725	194	1,693	291	541	180
2	523		894		1,158		501	
	266		694		1,658		590	
	99	98	186	210	261	298	170	246
	95		186		350		183	
	113		205		325		178	
	88	100	200	352	228	279	190	399
	125		190		287		247	
	81		231		313		233	
	75	134	196	1,759	292	490	247	1,226
	121		224		300		257	
3	117		340	87	282	333	393	226
	143	137	352		316		388	
	151		340		234		402	
	127	149	379	2,547	284	—	413	—
	124		1,821		466		1,273	
	150		1,698		514		1,179	
	127	534	1,806		498	211	1,245	
	147		1,712		482		1,207	
	168		64		401		211	
	110	534	79		336		246	
4	121		91		304		231	
	197		114		291		211	
	579	534	2,611	2,547	—	—	—	—
	549		2,718		—		—	
	475		2,305		—		—	
	535		2,556		—		—

Range and mean results of three separate cultures using one donor.

during the first day of culture and no DNA synthesis was evident at any time in lymphocytes treated with virus and subsequently inactivated. The uptake of thymidine cannot be attributed to viral activity in cultures treated with influenza, since this virus contains only RNA.

In two cultures in which inactivated influenza A2 virus was used to pretreat the stimulating lymphocytes the degree of thymidine incorporation was of the same order as when viable virus was used. This suggests that viral multiplication is not essential for the lymphocyte reactivity that we have observed, and it implies that the virus acts as an antigen. The supernatant from virus-treated lymphocytes which had been irradiated, however, caused no activation of fresh lymphocytes, though such supernatants might be expected to contain inactivated virus. The stimulatory capacity of the virus-treated lymphocyte cultures is therefore a function of the lymphocytes. We do not know whether this results from virus becoming attached to the cells or from membrane changes induced by virus.

Cells that had been only irradiated gave no significant counts when exposed to ^3H -thymidine.

Discussion

It has been shown that experimentally-induced viral tumours have surface antigens which will cross-react with one another (Klein, 1966). Similar changes are thought to occur in certain human tumours. For example, serum from a patient with Burkitt's lymphoma in remission has been shown to react specifically against the cells from others with this condition (Klein *et al.*,

1967). This suggests that there is a specific antigenic change in cells affected by the Epstein-Barr virus. Cultured glandular fever cells react with fresh autologous lymphocytes once the patient has recovered (Steel and Hardy, 1970), and since both these conditions are associated with viruses which have rather similar properties (Henle *et al.*, 1968) it appears likely that there is alteration of cell surface antigens due to the presence of this organism.

The findings presented here indicate that common viral infections can alter the surface characteristics of lymphocytes so that there is reactivity between affected and unaffected cells from the same individual. We do not know if this response is due to the virus becoming attached to the lymphocytes, or to an internal change with the formation of neoantigens, or to the presence of virus in the structure of the cell membrane.

Inactivated virus causes the same reactivity; thus whatever the underlying mechanism the change is determined by the lymphocytes themselves, as the subsequent stimulation of the autologous cells occurs without proliferation of the virus. Anomalous results in mixed cell cultures might well be caused by virally induced changes though such involvement was thought unlikely to explain the enhanced MLC reactivity caused by certain cell lines, some of which are known to be contaminated with virus (Hardy *et al.*, 1969; Hardy *et al.*, 1970).

Our observations may well be relevant to the progress of certain renal allografts. Hyperacute rejection has occurred after the transplantation of a kidney from an HL-A identical sibling suffering from a viral infection (H. Festenstein, personal communication), while viral infection in the host has been associated with episodes of accelerated rejection (Lopez *et al.*, 1973). It is not only viral infections that influence the course of renal allografts, for in dogs accelerated rejection occurs after pretreatment of the host with group A streptococci (Rapaport *et al.*, 1971). The influence of infection on the immunological status of a potential donor or recipient may well relate to the subsequent fate of any graft. Such responses might explain instances of rapid rejection which sometimes unexpectedly occur. It seems sensible to delay transplantation between identical siblings if the donor has even a mild viral infection.

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