Expression of OKM5 Antigen on Human Keratinocytes in vitro upon Stimulation with γ-Interferon

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Using murine monoclonal antibodies against human OKM5, OKM1 and HLA-DR antigens antigenic characteristics of freshly separated human epidermal cells (EC) and those of EC cultured in the presence of Interferon-γ (IFN-γ) were studied. After 8-12 days of culture, primarily OKM1- OKM5- HLA-DR- keratinocytes displayed OKM5 and HLA-DR antigens when exposed to IFN-γ. Our data support the concept, that human keratinocytes may possess accessory cell functions. (Received April 15, 1986.)

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Human epidermal cells (EC) are potent stimulators of antigen-specific allogenic T cell activation. This functional property depends on the presence of class II alloantigen bearing Langerhans’ cells (LC) (1). Ample evidence exists that in a wide variety of dermatoses keratinocytes bear HLA-DR surface moieties (2, 3). It has also been published that γ-interferon (IFN-γ) is capable of inducing HLA-DR synthesis and expression on cultured normal human keratinocytes (4, 5).

Aiming to confirm that keratinocytes may act as immunocytes we studied the phagocytosis of Candida albicans by separated human EC electronmicroscopically. Our investigations revealed that these cells are able to intake and kill living microorganisms (6). Furthermore, Yoneda et al. observed that human cultured EC devoid of LC are potent stimulators in Concanavalin-A driven T cell proliferation (7).

Recently it was proved that the monocyte/macrophage (M/M) lineage—the most important accessory cell population—consists of two functionally distinct subsets characterized by OKM1 and OKM5 monoclonal antibodies (8). Therefore it seemed to be reasonable to investigate the expression of OKM1 and OKM5 antigens on human EC stimulated with IFN-γ in vitro. In the present study we yield evidence that cultured normal human EC stimulated with IFN-γ became OKM5+ HLA-DR+ cells.

MATERIAL AND METHODS

Cell cultures

EC were prepared from fresh surgical skin specimens of healthy volunteers and cultured as described by Eisinger et al. (9). Although the normal epidermis is composed of ontogenetically distinct cell types, i.e., keratinocytes, Langerhans’ cells, melanocytes and Merkel cells, the culture conditions chosen result in the selective growth and differentiation of keratinocytes. Cells obtained by trypsinization were plated in 2.5 ml tissue culture flasks at a density of 3.5×10⁶ cells/cm² and grown in Eagle’s minimum essential medium with Earle’s salts and 25 mM Heps buffer (Gibco Limited,

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Paisley, Scotland) supplemented with 10% heat-inactivated foetal calf-serum, antibiotic-antimycotic solution (100 U penicillin, 100 U streptomycin, 2.5 mg fungizone, Gibco), L-glutamine (Gibco) and non essential amino acids (Gibco). Human IFN-γ (Sigma Chemical Company, St. Louis, MO, USA) (500 IU/ml) was diluted in culture medium prior to addition to EC cultures. EC were incubated at 37°C in 5% CO₂-containing atmosphere. Non confluent, 8-12-day-old cultures were used for the study.

Single suspensions of freshly isolated and cultured EC were resuspended in 2% bovine serum albumin (Sigma) in phosphate-buffered saline, pH 7.2 (PBS) at 10⁴ cells/ml. Cytocentrifuged smears were prepared using a SHANDON SOUTHERN cytopsin.

**Monoclonal antibodies**

Following monoclonal antibodies were used: OKM1, OKM5, (Ortho Pharmaceutical Corp., Raritan, NJ, USA) and HLA-DR (Becton Dickinson, Mountain View, CA, USA).

**Indirect immunofluorescence**

Reactivity with monoclonal antibodies was made visible by means of the indirect immunofluorescence (IF) technique. Briefly, the smears were frozen at -70°C for at least 12-24 h. After thawing they were washed in PBS and incubated with different monoclonal antibodies at a dilution of 1:20 for 30 min at room temperature. The smears were subsequently washed in PBS and incubated with rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark) at a dilution of 1:20 (30 min, room temperature). This was followed by PBS-washing and by a 30 min incubation with fluorescein-isothiocyanate labelled swine antirabbit antibody (Dakopatts) diluted 1:20 with PBS. After staining the specimens were washed in PBS and examined with a Leitz Ortholux incident light fluorescence microscope. Identification of cells was performed by switching to conventional light. Five hundred cells were counted in each smear, and the percentage of IF positive cells was calculated. Control sections lacking monoclonal antibodies were processed as above.

**RESULTS**

When expression of cell surface markers was examined on cytocentrifuged smears of freshly separated single EC from surgical skin specimens of healthy volunteers, 2-6% of EC exhibited specific IF staining when exposed to HLA-DR antibodies. OKM1 and OKM5 positive cells were seen in less than 2%. On the other hand 50-85% of EC cultured...
in the presence of IFN-γ exhibited strong to moderate IF positivity to OKM5 (Fig. 1), and HLA-DR antibodies, while OKM1 positive cells were less than 1%.

DISCUSSION

The monoclonal antibody OKM1 recognizes cell surface antigens on most of the M/Ms, null cells and granulocytes (10, 11). The OKM5 antibody reacts with cell surface determinants present on the majority of human M/M lineages (8). Both OKM1⁺ and OKM⁻ OKM5⁺ M/Ms are able to present soluble antigens to helper T lymphocytes, furthermore the OKM1⁺OKM5⁺ M/M lineage is the predominant subset that triggers autologous mixed lymphocyte cultures (8). In the present study we could show that EC became OKM5⁺ HLA-DR⁺ when cultured in the presence of IFN-γ.

Recent studies suggest that human EC can synthesize and express HLA-DR alloantigens in different dermatoses and in vitro due to IFN-γ stimulation as well (2, 3, 4, 5). Furthermore, it was also stated, that in positive intracutaneous tests for delayed-type hypersensitivity in projection of the dermal inflammatory infiltrate EC displayed both OKM5 and HLA-DR antigens (12, 13). HLA-DR, and OKM5 antigens, however, are characteristic markers for M/M lineages (8). The fact that cultured normal human EC stimulated with IFN-γ express both HLA-DR (4, 5) and OKM5 antigens (Fig. 1), in the light of previous findings concerning phagocytosis of Candida albicans by human keratinocytes (6) and keratinocytes as potent stimulators in Concanavalin-A-driven T cell proliferation (7) as well, supports the concept that human keratinocytes may possess accessory cell functions.

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REFERENCES

LAV/HTLV-III Infection and Atopy: Serum IgE and Specific IgE Antibodies to Environmental Allergens

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Infection with the LAV/HTLV-III virus leading to the clinical diseases of lymphadenopathy syndrome (LAS) or acquired immune deficiency syndrome (AIDS) goes along with marked dysregulation of the immune system (1, 2). Apart from well-known cellular immune defects observed in vitro and in vivo (3), a number of abnormalities of the humoral immune response have been described: polyclonal B-cell activation leading to increased IgG and IgA production with rather weakened production of IgM and decreased response to neoantigens together with increased amounts of circulating immune complexes (1, 3, 4). There is little information on the role of IgE in this condition. This may be due to the fact that most laboratories tend to heat infected sera at 56°C for 30 min thereby destroying IgE antibodies.

PATIENTS AND METHODS

We studied 69 patients of our AIDS outpatient clinic for the presence of atopic diseases in their personal and family history as well as for total serum IgE values (measured by paper-radio-immunosorbent-test (PRIST), Pharmacia, Uppsala) and specific IgE-antibodies (radio-allergy-sorbent test (RAST) Pharmacia, Uppsala) to 19 common environmental allergens.

Three groups of patients were compared: group I: LAV/HTLV-III-negative healthy homosexuals (n=39); group II: LAV/HTLV-III-positive healthy homosexuals (n=10); group III: patients with LAS or AIDS (n=20), among them four cases with full-blown AIDS including Kaposi’s sarcoma.

RESULTS

As Table I shows, the highest IgE serum values and the highest frequency of positive RAST results were found in group I (31% of the patients showing specific IgE antibodies...