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Role of macrophages in T lymphocyte response to Candida allergen in man with special reference to HLA-D and DR

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SUMMARY

Activation of naturally sensitized human T lymphocytes to Candida allergen was studied using three HLA-D and DR heterozygote Japanese cells (Dw1.DR1/DYT.DR4, Dw12.DR2/DYT.DR4, DYT.DR4/DEn.DR blank) and four HLA-D and DR homozygote cells (Dw1.DR1, Dw12.DR2, DYT.DR4, DEn.DR blank). *In vitro* activation of T lymphocytes to Candida allergen was found to require the presence of autologous or allogeneic compatible HLA-Dw1.DR1 and Dw12.DR2 macrophages.

INTRODUCTION

The association between HLA-DHO (now called Dw12) and low responsiveness to tetanus toxoid in man (Sasazuki *et al.*, 1978) and the *in vitro* activation of T lymphocytes to PPD requires the presence of autologous or allogeneic HLA-D compatible macrophages (Bergholtz & Thorsby, 1977, 1979). We reported the association between HLA-Dw1 and high responsiveness to Candida allergen in man (Nose *et al.*, 1980). In the present study we investigated the necessity of HLA-D compatible macrophages for the activation of T lymphocytes to Candida allergen.

MATERIALS AND METHODS

Materials. Three Japanese heterozygote cells (K.T., K.S. and K.K.) and seven HLA-D homozygote typing cells (HTCs) submitted to the 8th International Histocompatibility Workshop (8w101, 8w403, 8w401, 8w404, 8w402 and 8w405, without HOR) were tested for cell interaction in lymphocyte activation to Candida allergen. In three heterozygote cells, HLA-D and DR antigens were identified; K.T. (Dw1.DR1/DYT.DR4), K.S. (Dw12.DR2/DYT.DR4) and K.K. (DYT.DR4/DEn.DR blank), using 240 HLA-DR antisera of the 8th International Histocompatibility Workshop and HLA-D HTCs Dw1, Dw12 (DHO), DYT and DEn (Tsuji *et al.*, 1978). The same study was performed between the seven HTCs (8w101, 8w403-Dw1.DR1, 8w401, 8w404-Dw12.DR2, 8w402-DYT.DR4 and 8w405, HOR-DEn.DR blank). *Candida albicans* allergen extract was obtained from the Torii Chemical Company Limited, Tokyo.

Lymphocyte isolation. Lymphocytes were isolated from peripheral blood with Lymphoprep (Nyegaard Company) according to the method of Böyum (1964).

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Preparation of 2% neuraminidase-treated sheep red blood cells (NSRBCs). SRBCs were washed three times with RPMI 1640, 0·4 ml of packed SRBCs were resuspended in 5 ml of RPMI 1640 in the presence of 0·2 mg neuraminidase (1,000 units/ml), incubated for 30 min at 37°C, and washed twice with RPMI 1640 containing absorbed fetal calf serum (FCS) (20%) to give a 2% solution.

Absorbed FCS. FCS (GIBCO) was heat-inactivated (56°C, 30 min) and one volume of SRBC was added to two volumes of FCS. The mixture was incubated at 37°C for 30 min, and then at 4°C overnight. FCS was subsequently removed by centrifugation (10 min, 2,000 r.p.m.) and filtered through a 0·45-μm millipore filter.

Plastic dish coating. The plastic dish (6 cm in diameter, Corning Company) was filled with 3 ml of heat-inactivated, pooled AB-type serum, and incubated for 24 hr at 4°C. The dish was washed three times with RPMI 1640 before use.

Separation of T and B lymphocytes and macrophages. One volume of the 2% NSRBC solution was added to an equal volume of lymphocyte suspension. This mixture was layered over a Ficoll-Hypaque (RI 1·3545) gradient, incubated for 30 min at 4°C and spun down at 600 ***g*** for 12 min. The interface was removed and washed three times in RPMI 1640. Non-E rosette-forming cells were resuspended in complete medium (RPMI 1640+20% AB serum+6 mg kanamycin+1% L-glutamine) at a maximum concentration of 3×10^6 cells and incubated in a plastic petri dish for 60 min at 37°C in CO_2. The non-adherent cells were used as B lymphocytes after three washings with RPMI 1640, tested for purity (EAC rosette-forming > 95%) and resuspended in complete medium (5×10^5/ml). After removing the non-adherent cells, elution medium (0·2% EDTA+5% AB serum+RPMI 1640) was added, the adherent cells were collected by vigorous pipetting, washed three times in RPMI 1640, and resuspended in complete medium (5×10^5/ml). These cells were used as macrophages (peroxidase stain-positive cells > 90%).

E rosettes in the pellet at the bottom of the density gradient were resuspended in hypotonic RPMI (RPMI : distilled water = 1 : 2·6) and immediately spun at 1,000 ***g*** for 30 min. The cells were then washed three times with RPMI 1640 and resuspended in complete medium. These cells were used as T cells after inspection for purity (E rosette-forming cells > 95%).

Test for cell activation by Candida allergen. Cells (5×10^4/well) were cultured for 7 days in microculture plates (NUNC) in the presence of 50 μg Candida allergen. ^{3}H-thymidine (1 μCi) was added and 24 hr later the cells were harvested and counted in a scintillation counter. All tests were performed in triplicate and the c.p.m. of the mean value were calculated.

RESULTS

Activation of cell interaction by Candida allergen

Fig. 1 shows the activation of K.T. cells (Dw1.DR1/DEn.DR blank) by Candida allergen. Purified T lymphocyte, B lymphocyte and macrophage populations were not activated by Candida allergen. The addition of macrophages, but not of B cells, restored the response of purified T lymphocytes.

Effect of the number of macrophages on T cell activation

The relationship between T lymphocyte activation by Candida allergen and the number of macrophages is shown in Fig. 2. A log-linear relationship between the number of macrophages from 1×10^3 to 5×10^4/well (log 3·0–4·0), and an increase in T cell activation (log 4·0–4·5) were observed.

Fig. 3 shows the activation by Candida allergen of different cell populations from donors K.T. (Dw1.DR1/DYT.DR4), K.S. (Dw12.DR2/DYT.DR4) and K.K. (DEn.DR blank/DYT.DR4). Cells that had Dw1 or Dw12 (donors K.T. and K.S.) gave a high response to Candida allergen; a low response was observed in K.K. cells which had DYT and DEn, indicating that DYT is associated with low T cell responsiveness to Candida allergen.

Fig. 4 illustrates the role of HLA-D antigens in T lymphocyte activation by Candida allergen.

First, four types of HLA-D HTCs (Dw1, Dw12, DYT and DEn) treated with 40 μg/ml mitomycin (37°C, 40 min) were added independently to T lymphocytes from K.T. (Dw1.DR1/DYT.DR4). T lymphocyte activation was observed only in the presence of HLA-Dw1.DR1 HTC. Similarly, purified T cells from K.S. (Dw12.DR2/DYT.DR4) were only

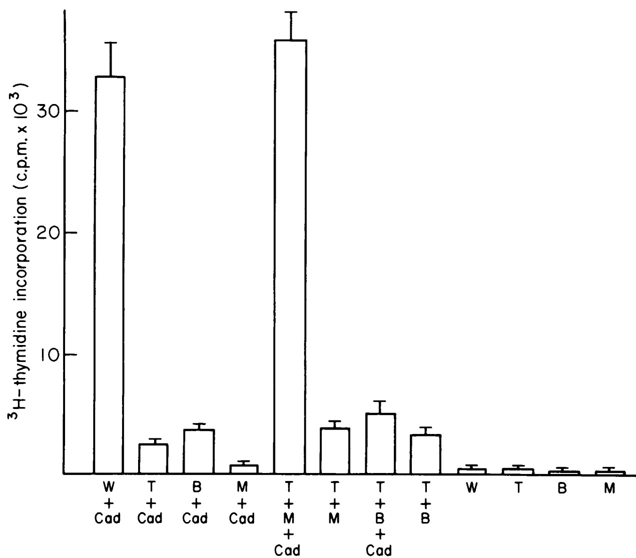

Fig. 1. Lymphocyte (K.T. cells) activation by Candida allergen. K.T. cell = HLA-Dw1.DR1/DYT.DR4, T = T lymphocytes (T > 95%, B < 5%, M = 0), M = macrophages (T = 0, B < 10%, M > 90%), Cad = Candida allergen, W = whole lymphocyte population, B = B lymphocytes (T < 5%, B > 95%, M = 0).

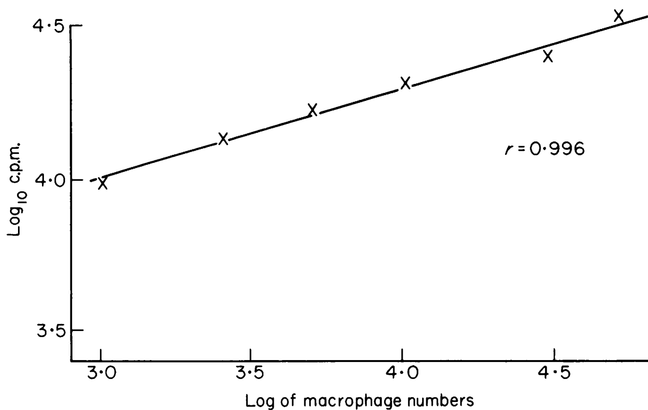

Fig. 2. Activation of T lymphocytes (K.T. cell) by Candida allergen in the presence of varying numbers of autologous macrophages.

activated in the presence of HLA-Dw12-, DR2-positive cells, while no activation was observed with cells from the low responder, K.K. (DEn.DR blank/DYT.DR4).

To establish the necessity for the presence of common D and DR antigens on macrophages and responding T cells, two experiments were carried out using the two D HTCs (Dw1 and Dw12) that gave high responses to Candida allergen (Fig. 3). Fig. 5 shows the response of T cells from D HTC 8w101 (Dw1, DR1) in the presence of several types of macrophages: 8w101, 403 (Dw1), 404, 401 (Dw12), 402 (DYT), HOR and 405 (DEn). The T cells were only activated in the presence of autologous macrophages or allogeneic macrophages carrying the Dw1 antigen but not in the presence of any other type of macrophages.

Fig. 6 shows the results of a corresponding study in the Dw12 series. Here also T cells from D HTC 8w404 (Dw12, DR2) were activated only in the presence of autologous macrophages or allogeneic macrophages, 8w401, carrying the Dw12 antigen.

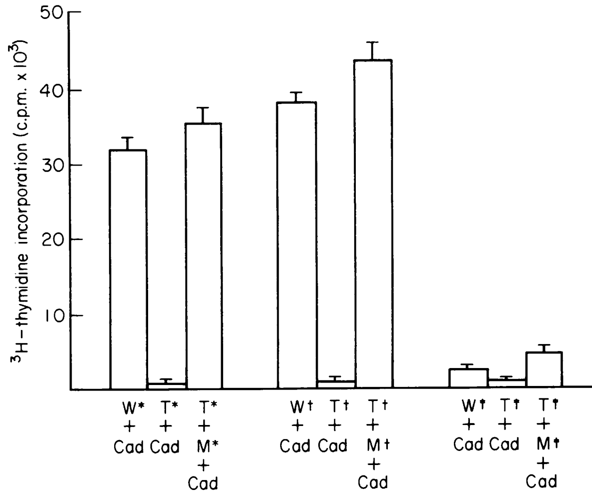

Fig. 3. Lymphocyte activation by Candida allergen. * K.T. cell = HLA-Dw1.DR1/DYT.DR4, † K.S. cell = HLA-Dw12 (DHO). DR2/DYT.DR4, ‡ K.K. cell = HLA-DEn.DR blank/DYT.DR4. W = whole lymphocytes, M = macrophages (T = 0, M > 90%), T = T lymphocytes (T > 95%, M = 0) Cad = Candida allergen.

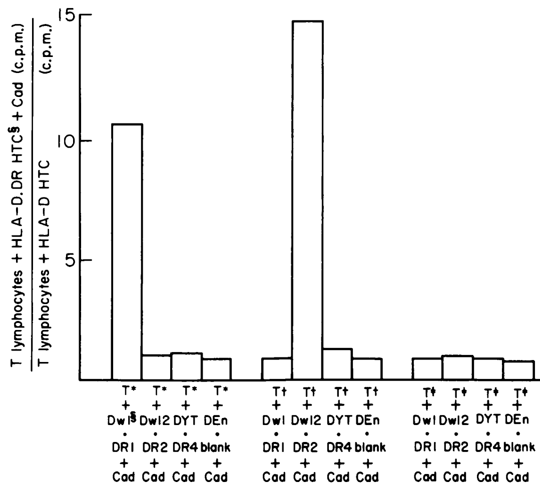

Fig. 4. T lymphocyte (K.T., K.S. and K.K. cell) activation by Candida allergen in the case of agreement of HLA-D and DR antigen. * K.T. cell = HLA-Dw1.DR1/DYT.DR4, † K.S. cell = HLA-Dw12 (DHO).DR2/-DYT.DR4, ‡ K.K. cell = HLA-DEn.DR blank/DYT.DR4, § Mitomycin-treated HLA-D.DR HTC.Cad = Candida allergen.

DISCUSSION

T lymphocyte responsiveness to PPD in guinea-pigs (Paul & Benacerraf, 1977) requires the participation of macrophages which share a major histocompatibility antigen with the responding T cells.

The secondary response to Streptococcus has been reported to be associated with HLA-B5 (Greenberg, Gray & Yunis, 1975). Furthermore, a strong correlation has been observed between HLA-Dw1 and the secondary response to Candida allergen (Nose *et al.*, 1980).

The present results demonstrate HLA-D-restricted macrophage participation in T lymphocyte activation by Candida allergen in man. These results are in agreement with those reported for PPD

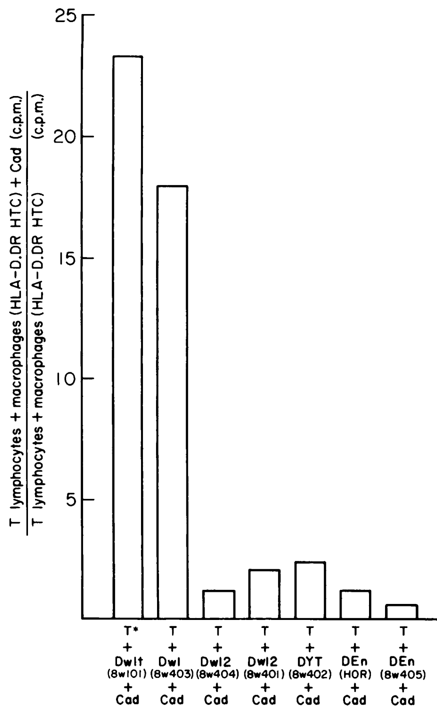

Fig. 5. T lymphocyte (8w101) activation by Candida allergen in the case of agreement of HLA-D and DR antigens. * T lymphocytes of HLA-Dw1.DR1 (8w101), † macrophages of HLA-D.DR HTC (8th International Workshop HLA-D.DR HTC in 8w101, 8w403, 8w404, 8w401, 8w402 and 8w405, without HOR). Cad = Candida allergen.

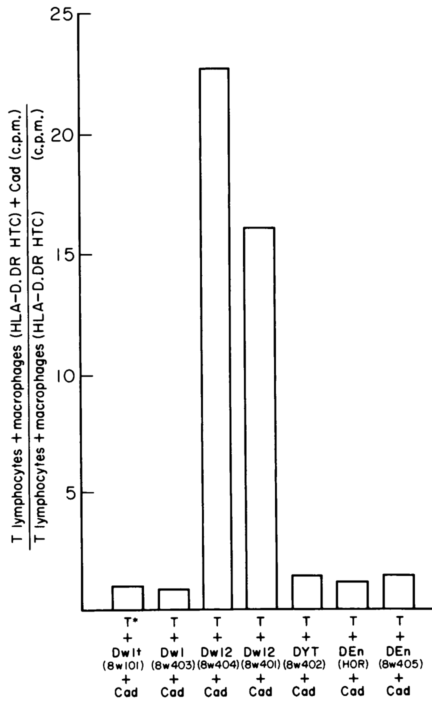

Fig. 6. T lymphocyte (8w404) activation by Candida allergen in the case of agreement of HLA-D and DR antigens. * T lymphocytes of HLA-Dw12.DR2 (8w404), † macrophages of HLA-D.DR HTC (8th International Workshop HLA-D.DR HTC in 8w101, 8w403, 8w404, 8w401, 8w402 and 8w405, without HOR). Cad = Candida allergen.

(Bergholtz & Thorsby, 1977). We also observed that HLA-Dw1 and Dw12 antigens were associated with strong activation, while HLA-DEn-positive T cells could not be activated by Candida allergen. At present, it is unknown how MHC-restricted T lymphocyte activation by Candida allergen is related with the disease mechanism(s) brought into play by Candida as a pathogen. To further our knowledge on this point, this type of study should be performed in human chronic candidiasis. This, the first report on the HLA-linked immune response to Candida allergen, strongly suggests the existence of Ir gene controlled immunoresponsiveness in the HLA-D and DR region.

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