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HLA Class II Restriction Specificity for Nickel-reactive T Lymphocytes

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Nickel-specific T cells were primed in vitro and restimulated with nickel in combination with autologous or allogeneic antigen-presenting cells. We have previously shown that not only DR, but also DQ molecules may take part in this process. To investigate this phenomenon further we applied recently available knowledge based on studies using specific cDNA probes and Restriction Fragment Length Polymorphism (RFLP), which has revealed a new level of DQ polymorphism, to search for a correlation of this polymorphism in the function of nickel-reactive T lymphocytes. We found that DQ, and presumably also DR, may restrict the proliferative response to nickel. The capacity of various antigen-presenting cells to cause restimulation correlated well, though not completely, with the specificity DQw1. RFLP splits of DQw1 did not yield any additional information on restriction specificity. Experiments using anti DR, DQ and DP monoclonal antibodies were performed, showing similar blocking capacity in all three antibodies. Work with cloned T cells is in progress to obtain more clearcut results concerning the HLA class II restriction of the proliferative T lymphocyte response to nickel. (Received October 11, 1987.)

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Specific T lymphocytes recognize antigen in connection with HLA molecules on the surface of Langerhans' cells (for review, see 1). This phenomenon is the basic mechanism for antigen presentation and may be responsible for the fact that certain diseases, such as HLA Cw6 in psoriasis (2), are HLA associated. The polymorphic HLA class II molecules are encoded by three distinct loci termed DR, DQ and DP. We have previously shown that the response of nickel-reactive T lymphocytes may be restricted not only by DR but also DQ (3). DQ typing is conventionally performed by serological methods and so far only three products, DQw1, DQw2 and DQw3, have been demonstrated (4). However, Restriction Fragment Length Polymorphism (RFLP) adds a level of polymorphism which corresponds to the DQ-genes (5–7). The purpose of the present investigation was to study the influence of this polymorphism in the DQ locus on the proliferative response of nickel-specific T lymphocytes.

MATERIAL AND METHODS

Cell donors: A 54-year-old female clerk with a previous history of hand eczema, anamnestic report of metal sensitivity and positive patch test against nickel sulphate was studied (Finn chamber) (8). She fulfilled the in vitro criteria for nickel allergy (9). Healthy individuals of a well-defined HLA-typed panel served as donors of antigen-presenting cells.

Tissue typing: HLA-A-B and C were determined with the lymphocytotoxic microtechnique (10). HLA-DR and DQ typings were performed on B-enriched lymphocytes (11). Ninth Workshop sera and local standard sera were used. HLA-DP typing was performed by Dr Nancy L. Reinsmoen (12), using the PLT (Primed Lymphocyte Typing) assay.

RFLP (Restriction Fragment Length Polymorphism) analysis: Southern blots of TaqI-digested genomic DNA were probed with either DRβ, DQα or DQβ cDNA as described previously (7). The DRβ, DQα and DQβ allelic patterns were classified according to earlier observations (7).

Cell culture medium: RPMI-1640 (Gibco Ltd, Paisley, Scotland) with 20 nM HEPES. Glutamine, penicillin, streptomycin and 10% inactivated pooled human serum were added.

Preparation of nickel sulphate and PHA: A stock solution of 1% $NiSO_4(6H_2O)$ (Merck, Germany) was prepared in 0.9% sodium chloride solution and then diluted in medium giving a final concentration of 25 µg/ml $NiSO_4$ in primary and secondary cultures. Phytohaemagglutinin (Wellcome) was used at a concentration of 50 µg/ml.

Mixed lymphocyte culture supernatants: (MLC-SUP) were prepared *ad modum* Schendel & Wank (13) and used as a source of IL-2, and activity was tested using the CTLL-2 cell line.

Cell preparation: Peripheral blood mononuclear cells (PBMC) were prepared from peripheral heparinized blood by flotation over Lymphoprep™ (Nyegaard, Oslo, Norway) (14). Antigen-presenting cells were prepared as described earlier (3). Briefly, purified mononuclear cells were incubated for 90 min in a 200 ml tissue culture flask (Nunc, Denmark). After three washings they were incubated for a further 18 h to allow the cells to adhere. Ice-cold ethylendiaminetetracetic acid (EDTA) (3.3 mg/ml) was added to the cells which were then left to stand for 45 min. The incubate was then shaken vigorously and rinsed to loosen adherent cells. A good purification of monocytes was obtained, since over 90% ingested Candida.

Nickel-reactive T lymphocytes were prepared by priming the PBMC (10^6/ml in 20 ml of medium) with 25 µg/ml $NiSO_4$ for 7 days in 50-ml tissue culture flasks (Nunc, Denmark). The cells were maintained at 37°C in humidified air containing 5% CO_2. After washing, the cells were resuspended and nickel-specific blasts were purified by Percoll™ (Pharmacia Fine Chemicals, Uppsala, Sweden) (15). The blasts were kept in mixed lymphocyte culture supernatant rich (25%) medium for 6 more days and then enriched for T-cells by rosetting with AET-treated sheep red blood cells (16).

Restimulation and assay of DNA synthesis: Cultures were set up in round-bottomed microtitre plates (Nunc, Denmark) in triplicate, each well containing 2.5×10^4 T-enriched blasts in 0.1 ml medium. 0.1 ml of medium (unstimulated cultures), or 10^4 irradiated (2000 rads) autologous or allogeneic macrophages, with or without $NiSO_4$ in the medium, was added. The plates were incubated at 37°C in humidified air

Table I. *Listing of the HLA types and responses of 2.5×10^4 nickel reactive T cells to nickel sulphate (25 µg/ml) upon restimulation for three days with 10^4 irradiated autologous or allogeneic macrophages followed by 18 h of tritiated thymidine incorporation*

The response was considered positive in the allogeneic combinations when the response exceeded 50% of the autologous combination. aut = autologous; allo = allogeneic; ND = Not Done

	DR	DQ_w	DP_w	DQβ	Response
BF-aut	2, w10	1	ND	I/II	Pos.
RG-allo	1, 2	1	ND	I/II	Pos.
HS-allo	1, 4	1, 3	2, 3	I/IV	Pos.
OR-allo	1, 1	1	1, 4	I	Pos.
LA-allo	3, 7	2	1, 2	III/VI	Neg.
TS-allo	2, 7	1, 2	ND	II/IV	Pos.
HN-allo	2, w11	1	2, 4	I/IV	Pos.
EM-allo	4, w13	1, 3	3, 5	IV/I	Neg.
LM-allo	1, w13	1	1, 5	I/II	Pos.
JM-allo	4, w8	3	2, 3	IV	Neg.
JW-allo	3, 7	2	ND	III/IV	Neg.
SH-allo	1, 4	1	2, 4	I/V	Pos.
ASB-allo	4, w13	1, 3	ND	II/V	Pos.
BC-allo	4, w13	1, 3	3, 4	V/I	Pos.
SSP-allo	4, w13	1, 3	3	II/V	Pos.

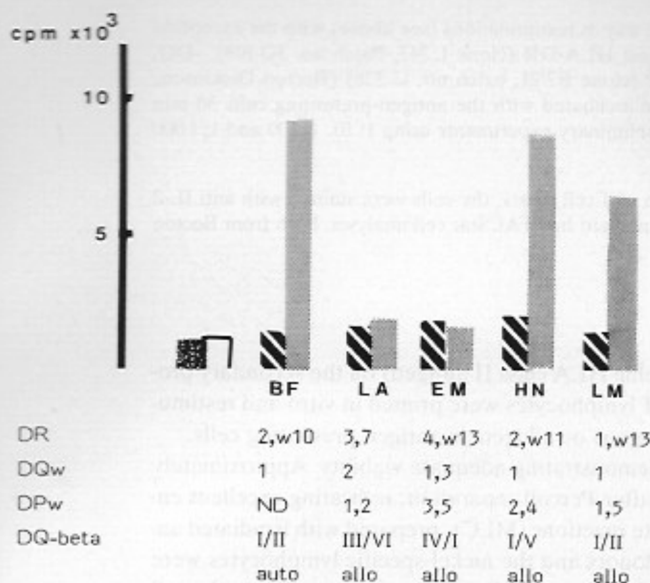

Fig. 1. Responses of 2.5×10^4 nickel reactive T lymphocytes from a patient (BF) to $NiSO_4$, 25 µg/ml upon restimulation for 3 days with 10^4 autologous and allogeneic macrophages. □, Nickel-specific lymphocytes + medium; ▦, nickel-specific lymphocytes + nickel + adherent cells; ▧, nickel-specific lymphocytes + adherent cells; ▩, nickel-specific lymphocytes + nickel sulphate.

containing 5% CO_2 for 3 days. One µCi of [methyl-^{3}H]-thymidine (Amersham International Plc, Amersham, England, specific act. 5 Ci/mmol), in a volume of 0.05 ml, was added to each well 18 h before harvesting was carried out on fibreglass filters with a Titertech cell harvester (Flow Labs., Irvine, Scotland). Filters were dried and assayed in an LKB liquid scintilation counter (Wallac, 1216 Rackbeta II, Finland).

Results were expressed as mean counts per minute (cpm) and relative response, i.e. results from all experiments were pooled and the responses in each experiment were related to the response of autologous antigen presenting cell in the presence of nickel sulphate, which was considered to be a 100% response.

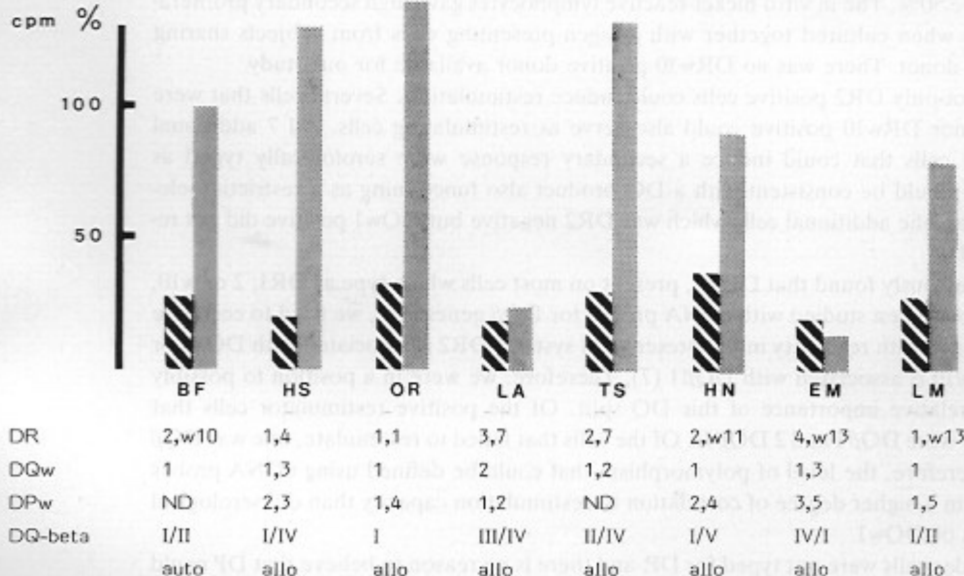

Fig. 2. Same as Fig. 1, but here the responses are expressed in relative counts per min, i.e. pooled responses from four experiments and the responses of the allogeneic macrophage combinations are related to the response of the autologous combination.

Blocking experiments were performed in the same way as restimulations (see above) with the exception that specific monoclonal antibodies directed against HLA-DR (clone L 243, batch no. JO 103), -DQ, (Leu 10, clone SK 10, batch no. JO 105) or -DP (clone B7/21, batch no. C 326) (Becton Dickinson, Mountain View, Calif., USA) diluted 1: 100 were incubated with the antigen-presenting cells 30 min prior to culture. This dilution was chosen after preliminary experiments using 1: 10, 1: 100 and 1: 1 000 in the autologous cell combination.

Analysis of activated T-cells: After the purification of T-cell blasts, the cells were stained with anti IL-2 receptor monoclonal antibodies (anti-TAC) and analysed in a FACStar cell analyser, both from Becton Dickinson (Mountain View, Calif.).

RESULTS

In order to study the impact of the polymorphic HLA class II antigens on the secondary proliferative response to nickel, nickel-specific T lymphocytes were primed in vitro and restimulated with nickel in combination with autologous or allogeneic antigen-presenting cells.

The blasts gave high responses to PHA, demonstrating adequate viability. Approximately 95% of the blasts expressed IL-2 receptors after Percoll separation, indicating excellent enrichment of activated cells. Mixed lymphocyte reactions (MLC), prepared with irradiated antigen-presenting cells from the stimulating donors and the nickel-specific lymphocytes were negative, indicating the loss of alloreactivity in this model. In contrast, non-tranformed small T cells from the nickel-sensitive donor gave low antigen-specific secondary responses on day 3 of culture but high MLC to the allogeneic antigen-presenting cells on day 6. This means that the non-stimulatory allogeneic cells, prepared as described above, had still not lost their alloreactivity.

The results of the tissue typing, and a summary of the responses with these cells in several experiments are shown in Table I. Typical experiments are shown in Figs. 1 and 2. All reactions resulting in ≥50% of the response in the autologous combination were considered to be positive. The experiments were repeated 3–4 times. In 3 out of 4 cases the positive allogeneic responses were in the region of 80–90% of the autologous, while the remainder were just above 50%. The in vitro nickel-reactive lymphocytes gave high secondary proliferative responses when cultured together with antigen-presenting cells from subjects sharing DR2 with the donor. There was no DRw10 positive donor available for our study.

However, not only DR2 positive cells could induce restimulation. Several cells that were neither DR2 nor DRw10 positive could also serve as restimulating cells. All 7 additional DR2 negative cells that could induce a secondary response were serologically typed as DQw1, which would be consistent with a DQ product also functioning as a restriction element. However, one additional cell, which was DR2 negative but DQw1 positive did not restimulate (EM).

Since we previously found that DQw1, present on most cells which type as DR1, 2 or w10, is heterogeneous when studied with cDNA probes for DQβ genes (17), we tried to correlate this genomic split with reactivity in the present test system. DR2 is associated with DQβI or II, while DRw10 is associated with DQβI (7). Therefore, we were in a position to possibly evaluate the relative importance of this DQ split. Of the positive restimulator cells that lacked DR2, 5 were DQβI and 2 DQβII. Of the cells that failed to restimulate, one was DQβ I positive. Therefore, the level of polymorphism that could be defined using cDNA probes did not result in a higher degree of correlation to restimulation capacity than did serological determination of DQw1.

The responder cells were not typed for DP, and there is no reason to believe that DP could not serve as a restriction element in these experiments. However, we found no clear correlation of DP phenotype between positive and negative stimulator cells.

Since the enriched cells in the present bulk cultures might contain a mixture of different

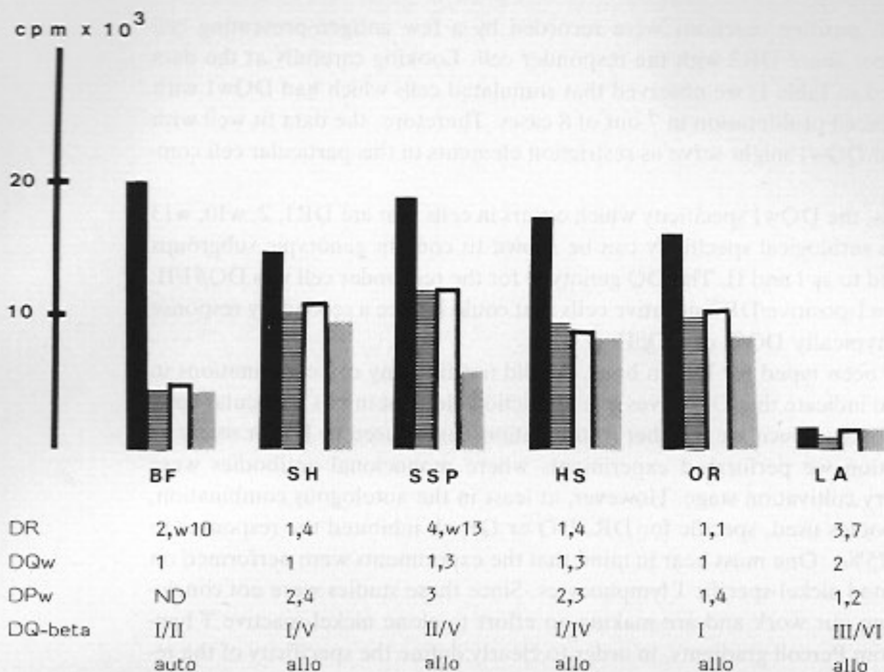

Fig. 3. Same as Fig. 1, but here monoclonal antibodies (moab) directed against DR, DQ or DP-molecules were added to the cell suspensions 30 min before culture. ■, Nickel-specific lymphocytes + nickel + macrophages + medium; ▦, nickel-specific lymphocytes + nickel + macrophages + moab anti DP; □, nickel-specific lymphocytes + nickel + macrophages + moab anti-DR; ▤, nickel-specific lymphocytes + nickel + macrophages + moab anti-DQ.

T cell clones with distinct restriction elements, we tried to define the major restimulatory class II antigens by blocking with DR, DQ and DP specific monoclonal antibodies. However, we found that all these reagents resulted in an inhibition of approximately 45–75% of responses, and our experiments were therefore, not conclusive in this respect (Fig. 3).

DISCUSSION

The HLA-D region contains three distinct loci which encode the polymorphic class II molecules which are expressed in varying degree in different tissues (18). The epidermal Langerhans' cells carry HLA class II antigens and are more potent antigen-presenting cells than are blood monocytes (19). The polymorphism of the DR locus has been well documented using serological reagents, whereas the available polymorphism which can be elucidated using antibodies for DQ is limited, compared with the degree of polymorphism demonstrable by biochemical or genetic approaches. Paulsen et al. (20) have demonstrated an HLA-DR restriction element at the genomic level using RFLP and chlamydial antigens in a proliferation assay. Using the in vitro model described, we have an opportunity to study the relevance of the increased DQα and DQβ polymorphism, obtained at the DNA-level (RFLP), in the interactions between T-lymphocytes and antigen-presenting cells. We selected a panel of cells from donors who had been carefully typed using cDNA probes as well as serological reagents. We found that restimulation of nickel-reactive lymphocytes from a donor with a DR-type 2, w10, DQw1 were restimulated by donors who were DR2-positive. In our panel of stimulator cells there were four DR2-positive cells that all induced a positive

response. In addition, positive reactions were recorded by a few antigen-presenting cell populations that did not share DR2 with the responder cell. Looking carefully at the data (which are summarized in Table I) we observed that stimulated cells which had DQw1 with the responder cell induced proliferation in 7 out of 8 cases. Therefore, the data fit well with the idea that DR2 and DQw1 might serve as restriction elements in this particular cell combination.

Using cDNA probes, the DQw1 specificity which occurs in cells that are DR1, 2, w10, w13 and w14 positive, the serological specificity can be shown to contain genotypic subgroups which we have referred to as I and II. The DQ genotype for the responder cell was DQβI/II. The serologically DQw1-positive/DR2-negative cells that could induce a secondary response where all either genotypically DQβI or DQβII.

Our donor has not been typed for DP. In brief, we did not find any cell combinations in this study which would indicate that DP serves as a restriction element in this particular combination. In order to further elucidate whether restimulation was induced by DR or solely by DQ in this combination we performed experiments where monoclonal antibodies were added in the secondary cultivation stage. However, at least in the autologous combination, the monoclonal antibodies used, specific for DR, DQ or DP, all inhibited the responses to a similar degree (45–75%). One must bear in mind that the experiments were performed on bulked Percoll-separated nickel-specific T lymphocytes. Since these studies were not conclusive, we are continuing our work and are making an effort to clone nickel-reactive T lymphoblasts obtained from Percoll gradients, in order to clearly define the specificity of the restricting element for nickel reactivity.

The finding that nickel-proliferative responses could primarily be DQ-restricted is interesting and might be coupled with the association of particular DQ types with allergic T cell mediated responses to nickel. Such studies are presently in progress.

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