Brief Definitive Report

Corecognition of HLA-A1 and HLA-DPw3 by a Human CD4+ Alloreactive T Lymphocyte Clone

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Summary

We have generated an alloreactive proliferative T cell clone that only is stimulated by HLA-DPw3+ antigen presenting cells (APC) that at the same time carry HLA-A1. The T cell clone is CD4+, and the proliferation is blocked by anti-DP monoclonal antibodies and not by antibodies towards other class II or towards class I molecules. Family studies show that APC with A1 and DPw3 on different haplotypes (*trans*) are able to stimulate the clone, and an HLA recombinant family gives evidence that the class I–carrying part of the haplotype is necessary for stimulation to occur. Stimulation is also observed with mixtures of APC expressing DPw3 and APC expressing A1, and likewise, DPw3+ APC become stimulatory when preincubated with supernatants from A1-positive cells. Our studies suggest that major histocompatibility complex (MHC) class I peptides presented by class II are allostimulatory and that APC can process MHC molecules that presumably are presented as allele-specific peptides in the context of other MHC molecules. We hypothesize that presentation of MHC peptides by MHC molecules constitutes an important part of alloreactive phenomena in vivo and in vitro.

The MHC has a fundamental role for selection of the T cell repertoire. The TCR recognizes probably a complex consisting of an antigenic peptide and the MHC molecule on the APC (1, 2). It is commonly agreed that the alloreaction is comparable with the antigen-specific response, with the important modification that the peptide involved is a self peptide (originating from the APC or the immediate environment) presented by an allogeneic MHC molecule. Most of the self peptides presented are probably nonpolymorphic intra- or extracellular degradation products, but the minor histocompatibility antigen systems are examples of presentation of polymorphic self peptides. Other examples of presentation of polymorphic peptides have been found in experiments using transgenic mice or transfected cells where MHC peptides may modulate the alloreactive response (3–5).

We have studied a human alloreactive T cell clone that probably recognizes a class I peptide in the context of a class II molecule, and we hypothesize that presentation of MHC peptides by MHC molecules constitutes an important part of alloreactivity in vivo and in vitro.

Materials and Methods

Generation of T Cell Clones. PBMC from a responder "B" with type HLA-A2,25; B7,44; DR1,4; DQw1,3; DPw4 were cocultured with an equal amount of irradiated (20 Gray = 2,000 rad) PBMC from a donor "DL" HLA-A1,28; B7,8; DR1,4; DQw1,3; DPw3 (at 10^6 cells/ml) in culture medium (RPMI 1640 supplemented with 2 mM L-glutamine, 80 ng/ml streptomycin, 200 IU/ml penicillin, and 10% inactivated pooled human serum). After 6 d of culture in a humidified atmosphere at 37°C in 5% CO_2, the blasts were purified over a 35–45% Percoll gradient and cloned in round-bottomed microtiter plates (Nunc, Roskilde, Denmark) at 0.5 cells/well with 10^5 irradiated (100 Gray) lymphoblastoid cell line (LCL) cells from the original stimulator in a total volume of 150 μl of culture medium. 3 d later, 10% of T cell growth factor–rich supernatant (TCGF sup) was added. At day 12, the positive wells were transferred to 2-ml well plates and restimulated with 10^5 irradiated LCL from donor DL. The culture medium containing 10% TCGF sup was changed three times a week, keeping the cell concentration $< 5 \times 10^5$ cells/ml. Every 12 d, the clones were restimulated until sufficient amounts were obtained, whereupon the cloned cells were cryopreserved in liquid nitrogen at the end of restimulation cycles.

Proliferative Assays. The thawed clones were plated at 10^4 cells/well in round-bottomed microtiter plates in the presence of 2.5×10^4 irradiated (100 Gray) LCL cells or 5×10^4 irradiated (20 Gray) PBMC in a total of 150 μl of culture medium. After 48 h, the cultures were labeled with 1 μCi/well of [^{3}H]thymidine; 16 h later, the cells were harvested on glass fiber filter by a semiautomatic harvester (Skatron, Lierbyen, Norway), and the incorporation of labeled thymidine was quantitated by liquid scintillation spectrometry. All cultures were done in triplicates and the median counts per minute was used for evaluations.

Mixtures of APC. The irradiated (20 Gray) PBMC or (100 Gray) LCL from two stimulators were mixed in various proportions, and

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mixed cells (2.5×10^4 of LCL and 5×10^4 of PBMC) were incubated at various times at 37°C in 5% CO_2 in round-bottomed microtiter plates in 100 μl culture medium before addition of 10^4 clone cells in 50 μl of culture medium. The proliferative assay was then carried as described above.

mAb Inhibition Assays. Four mAbs were used: L243 that reacts with monomorphic determinants on DR molecules; TU22 (anti-DQ); B7/21 (anti-DP); and W6/32 (anti-HLA-class I). The blocking assay was carried out according to the 10th workshop protocol with at least four different fivefold dilutions (6).

Results and Discussion

Of 20 proliferative clones obtained from the priming (see above), six could be expanded to reasonable number in order to allow extended testings. The clone B/DL3, which is $CD4^+$, showed a peculiar restimulation pattern. In a panel (7) of lymphoblastoid cell lines (LCL) from the 10th workshop, it appeared that all stimulatory APC carried HLA-A1 in addition to the HLA-DPw3 antigen. This reactivity pattern was confirmed in a local panel, and >60 different stimulator cells have been tested repeatedly with similar results. The reactivity of a subclone obtained by limiting dilution was identical when tested on a small selected panel.

Testing in families showed that when A1 and DPw3 were present on different haplotypes, only cells from individuals that were at the same time A1 and DPw3 stimulated the clone. In the family shown in Fig. 1, one individual had a recombination between HLA-A and HLA-DR, and this provided evidence that the HLA class I carrying part of the haplotype was needed in order to give stimulation. The A3 and A11 antigens that are serologically crossreactive with A1 did not give any stimulation when present on $DPw3^+$ cells. Blocking experiments with mAbs showed that only anti-DP antibodies were able to block the stimulation; anti-DR, anti-DQ, or anti–class I antibodies had little effect (Fig. 2).

Mixing experiments were performed with APC carrying different combinations of class I antigens and DP antigens. Mixtures of cells being DPw3 and cells being A1 were indeed able to stimulate the clone and this in a dose-dependent way (Fig. 3). Preliminary experiments showed that a 4-h preincubation of the stimulating cells was optimal and that LCL as well as PBMC could be used as APC in this and the other experiments mentioned. A strong stimulation of the clone was obtained when $DPw3^+$ APC were preincubated with supernatant from an $A1^+$ LCL and not with supernatant from an $A1^-$ LCL (Table 1). This supernatant apparently contains a sufficient amount of A1 molecules, which after processing by the $DPw3^+$ APC, are presented as peptides on DPw3 molecules. We have tested two peptides specific for A1 (amino acids 65–84 and 143–158), but these peptides do not stimulate our clone when preincubated with $DPw3^+$ APC, and other peptides are under construction.

Several recent publications demonstrate that HLA class I peptides can be recognized when presented on class I molecules of another specificity (3) or that such peptides may inhibit alloreaction (4, 5). Likewise, viral peptides are able to inhibit alloreaction (8). Our findings suggest that the processing of class I peptides is a normally occurring in vitro phenomenon in APC and that class II molecules are able to present class I peptides.

Two earlier reports deal with $CD4^+$ T cell clones having a specificity correlating closely with a class I specificity, al-

Table 1. *Proliferative Responses of the Clone B/DL3 against LCL Incubated with Culture Supernatants*

Stimulators	Workshop no.	Responders					
		B/DL3			Control reagent		
		$A1^+$ sup	$A1^-$ sup	Medium	$A1^+$ sup	$A1^-$ sup	Medium
		cpm					
STEINLIN (A1,DPw3)	9057	10,982	8,634	11,445	38,325	39,895	31,430
SLE005 (DPw3)	9059	24,871	124	97	45,150	39,827	42,710
LOO81785 (DPw3)	9018	12,209	444	555	42,338	40,730	43,636
BM21 (A1)	9043	0	0	0	45,597	41,339	45,247
BOLETH	9031	0	0	0	22,795	20,982	24,954

The test shows that the clone is stimulated by $DPw3^+$ APC when these are preincubated with supernatant from $A1^+$ LCL ($A1^+$ sup). Figures are median counts per minute of triplicate cultures, experimental value minus the value of stimulator cells alone. A zero indicates that the experimental value is equal to or lower than that obtained with stimulator cells alone. The culture supernatants were from two LCLs, positive ($A1^+$ sup) and negative ($A1^-$ sup), respectively, for A1. The supernatant was collected after 3 d of culture at a concentration of LCL of $\sim 10^6$ cells/ml, filtered through a 0.22-μm filter, and 50 μl was added to the stimulator cells. After an incubation at 37°C for 4 h, the clone or control reagent was added and the proliferative test carried out as previously described. The HLA type of the stimulator cells: STEINLIN: A1,B8,DR3,Dw3,DQw2,DPw3/4; LOO81785: A3,24,B18,DR3,Dw3,DQw2,DPw3; SLE005: A2,B60,DRw13,Dw19, DQw6,DPw3; BM21: A1,B41,DRw11,Dw5,DQw7,DPw2; BOLETH: A2,B62,DR4,Dw4,DQw8,DPw4. The control reagent is a polyclonal T cell line raised against a pool of stimulators.

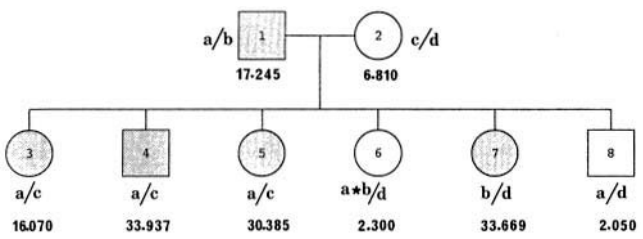

Figure 1. The proliferative responses of the clone B/DL3 against the LCL from an informative family. The figures are median counts per minute of triplicate cultures. Haplotypes in the family: a: HLA-A2, B17, DRw6, DQw1, DPw3; b: HLA-A1, B17, DR7, DQw3, DPw3; c: HLA-A1, B8, DR3, DQw2, DPw2; d: HLA-A9, B40, DRw6, DQw1, DPw2; a*b: HLA-A2, B17, DR7, DQw3, DPw3. A recombination between the A and DR locus on the chromosome inherited from the father was observed in the child no. 6. The cells from this child fail to stimulate the clone B/DL3. The presence of HLA-A1 and DPw3 at the same time coincides with a strong stimulation, this combination can be in *cis* (nos. 1 and 7) or *trans* (nos. 3, 4, and 5). A weak stimulation by the cells of the mother is probably due to the presence of A1 (see text).

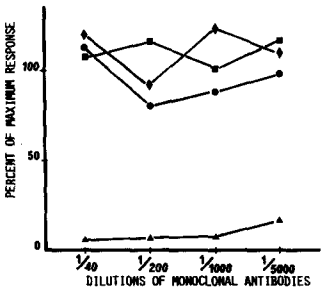

Figure 2. Proliferation of clone B/DL3 towards a A1,DPw3$^+$ LCL in presence of mAbs towards HLA. Antibodies used (6): (●) L243 that reacts with monomorphic determinants on DR molecules; (◆) TU22 (anti-DQ); (▲) B7/21 (anti-DP); (■) W6/32 (anti-HLA-class I). The response is percentage of response without antibody.

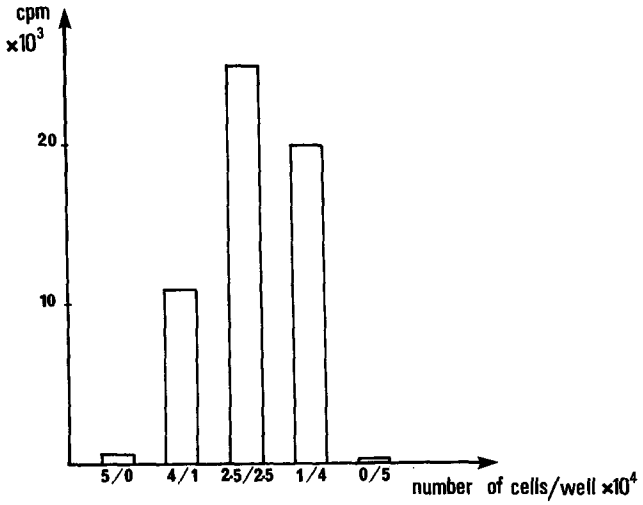

Figure 3. Proliferative responses of clone B/DL3 against mixtures of cells being A1 and DPw3. Each column represents the response of the clone towards cells from the donors A and B in varying proportions. The left-hand column represents the response of the clone towards cells from A only, and the right-hand column towards cells from B. Donor A: HLA-A1,29, B8,44, DR5,7, DPw2; donor B: HLA-A24,29, B27,44, DR3,w14, DPw2,3. Cell mixing from other panel donors or LCL from the workshop (>30 combinations tested) gave identical results. When A1 and DPw3 were present on the two cell populations, a stimulation was seen.

though the reaction was blocked by anti-class II antibodies and not by anti-class I antibodies (9, 10). In those cases, no restriction by class II was described, and this suggests that the presentation of class I peptide was on a class II molecule showing little polymorphism, or alternatively, that the same class I peptide could be presented by several class II molecules. In the latter case, the T cell clone presumably recognizes only the class I peptide and not the allodeterminants of the class II molecule (11). We have also, in some more recent experiments, observed that A1$^+$DPw3$^-$ APC that earlier did not stimulate could provoke a stimulation of the clone B/DL3, while A1$^-$DPw3$^+$ APC never have given stimulation. In mice, isolated class I differences cause a strong proliferation in primary mixed lymphocyte reaction (MLR), and the reaction can be blocked by antibodies towards responder Ia while MLR towards class II differences are blocked by antibodies towards Ia of the stimulator cells (12).

The mixing experiments and the positive effect of medium conditioned by A1-bearing cells suggest that class I HLA from one cell can be presented as a peptide on class II HLA molecules on another cell. This observation has possible implications for clinical transplantation, as APC from either donor or recipient may present allogeneic HLA peptides and thus initiate rejection processes.

T cell clones often display a reaction pattern that correlates closely with a specificity defined by other methods (6), and in such cases, the peptide presented by MHC is probably a nonpolymorphic peptide originating from an autologous protein that may be tissue specific (13). Other clones have reactions that are difficult to reconcile with a certain specificity or epitope, even with knowledge of amino acid sequences of the MHC molecule in question. Such reactions usually segregate with HLA haplotypes in families but may also appear to depend on two different HLA haplotypes (14). It is tempting to speculate that such atypical results are due to the presentation of a polymorphic MHC peptide, either class I or II, by another MHC molecule.

Our hypothesis also explains some curious results on T cell clones apparently stimulated by hybrid antigens (15, 16). One of these reports (15) deals with a clone that only was stimulated by cells that were at the same time DR2 and DR4. As the DRα chain is nonpolymorphic, it was assumed that the stimulatory molecule was a heterodimer consisting of a DRβ chain and a DQα chain. Although the existence of such molecules has been suggested in other cases by biochemical studies (17), the blocking studies performed by mAbs were incompatible with such hybrid heterodimers. A more plausible explanation is the presentation of a polymorphic DQ (or DR) peptide by a DR molecule.

A recent report (18) describes T cell clones reactive with a DR3 peptide presented by DPw3. This indicates that the same class II molecule (DPw3) has the possibility to bind different HLA peptides. Examples of presentation of class II

peptides by class I molecules have not yet been reported, but it can be speculated, as recently suggested by Janeway (19), that this might happen in T cells and constitute the molecular basis for T cell suppressor networks.

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