Heated lymphocytes express HLA-DR antigens despite their inability to stimulate in MLC

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We have utilized serological techniques and mixed lymphocyte culture (MLC) reactions to examine HLA-DR and HLA-D expression by heated (45°C for 1 h) lymphocytes in order to study the functional relationship of these antigens. Heated lymphocytes do not stimulate proliferation of allogeneic lymphocytes in MLC, yet they express HLA-DR antigens. The fraction of peripheral blood lymphocytes (PBL) expressing DR is not altered by heating, nor is the staining intensity altered as detected by fluorescence microscopy. Alloantiser to "B cell alloantigens" recognize HLA-DR determinants on heated cells without any detectable change in either specificity or quantitative cytotoxic effects. Flow cytometry with monoclonal antibody demonstrates only minimal decrease in HLA-DR expression after heating. Thus stimulation in MLC requires more of the stimulating cell than the mere expression of HLA-DR.

Received for publication 26 January, revised 28 April, accepted 13 May 1983

Foreign HLA-D antigens induce T-cell proliferation measured in the mixed lymphocyte culture reaction (MLC), and are present on a restricted range of tissue types (Bach et al. 1976, Hirschberg et al. 1976, Stingl et al. 1978). Lymphocytes given hyperthermic treatment in vitro (45°C for 1 h) are unable to activate proliferation of allogeneic T lymphocytes in an MLC reaction (Schellekens & Eijsvoogel 1970). This indicates they are functionally unable to express their HLA-D antigens. In contrast, heated lymphocytes present their HLA-A, -B, and -C antigens in an effective way to foreign T lymphocytes, provided a separate helper stimulus (either foreign HLA-D bearing cells or soluble antigens to which the donor responds in vitro) is added to activate a proliferative helper response (Eijsvoogel et al. 1973, Hank & Sondel 1982).

Abbreviations:

3H-TDR = Tritiated thymidine
HS-RPMI = RPMI supplemented with 15% human serum
MHC = Major histocompatibility complex
MLC = Mixed lymphocyte culture assay
PBL = Peripheral blood lymphocytes

* J.A.H. is a Fellow of the Cancer Research Institute, Inc., New York, NY.
** P.M.S. is a G. L. and J. A. Hartford Foundation Fellow and a Scholar of the Leukemia Society of America.

This work was supported by grant CH-237 of the American Cancer Society and R01-CA322685-01 of the N.I.H.
HLA-DR antigens are genetically controlled within the HLA-D region and detected on the same tissues as HLA-D (Natali et al. 1981). Despite identification of individuals inheriting what appears to be a haplotype bearing a D-DR recombinant (Mawas et al. 1980, Oksenberg et al. 1982, Suciu-Foca et al. 1980), the molecular relationship between HLA-D and HLA-DR antigens remains unclear. They may reflect the products of distinct closely linked genetic loci, or alternatively represent different domains on a single polypeptide. In mouse, recognition of I-A, (the HLA-D analogue) by proliferating T cells is inhibited by antibody to I-A (Germain et al. 1982). Recognition of HLA-D antigens in an MLC can be inhibited by anti HLA-DR antisera (Humphreys et al. 1976, Bergholtz et al. 1978). These findings suggest that the antigens recognized by T cells in MLC and those recognized by alloantisera are either on the same or very proximal cell surface molecules.

The present study examines the functional non-expression of HLA-D on heated cells, based on their inability to stimulate in MLC, and contrasts this to their continued expression of HLA-DR as determined by serological methods. Despite their inability to stimulate in MLC, heated lymphocyte preparations contain a normal fraction of HLA-DR bearing cells. Furthermore, their alloantigenic DR specificities are not altered as detected by cytotoxicity testing with alloantisera. Analysis by flow cytometry demonstrates a minimal (~20%) decrease in detectable HLA-DR antigen per cell following treatment with hypertermia.

Material and methods

Lymphocytes and media

From blood obtained from healthy paid volunteer donors, peripheral blood lymphocytes (PBL) were obtained by Ficoll Hypaque flotation. These were washed twice and resuspended in RPMI 1640 supplemented with 25 mM Hepes buffer, L-glutamine, penicillin, streptomycin, and 15% (vol./vol.) heat inactivated non-transfused human male serum (HS-RPMI).

Nylon wool enrichment for B lymphocytes

Lymphocytes (3 x 10⁷) were incubated on 1 g of sterile nylon wool in a 20 cc syringe for 60 min at 37°C. A population enriched in T lymphocytes was gently eluted off the nylon wool column. Forceful agitation and elution enabled the recovery of nylon adherent cells, which were enriched in B lymphocytes and monocytes (Handwerger et al. 1974).

In vitro hyperthermia treatment

Lymphocytes were suspended in 5 ml at 1 x 10⁹/ml and placed in 15 ml conical glass tubes. These were placed in a shaker water bath at 45°C for 60 min (unless stated otherwise). Temperature monitoring, done with a Digitel (5810 thermometer) in a control tube containing culture medium alone, enabled temperature accuracy to be maintained within 0.2°C. Three min were required for the 5 ml volume to attain 45°C under these conditions.

Mixed lymphocyte cultures

Fresh responding PBL (1.0 x 10⁵) were cocultured in MLC with irradiated (2500 R delivered by a cesium source) or hyperthermia treated stimulating lymphocytes (0.5 and 1.0 x 10⁵) in 0.2 ml HS-RPMI in round-bottom 0.25 ml microwells. After culturing for 6 days at 37°C in a 5% CO₂ atmosphere, 1 μCi of tritium labeled methyl thymidine (³H-TdR-New England Nuclear, Boston) was added in 0.05 ml. After 7 h of labeling, cultures were harvested for liquid scintillation counting using a MASH device (Otto Hille,
Data presented are means of quadruplicate cultures ± S.E.M.

Mitogen stimulation

Fresh PBL (1.0 × 10^5) were cultured in 0.2 ml HS-RPMI with 1% PHA-M (Difco, Detroit, Michigan). After 72 h, microwells were labeled for 7 h with ^3H-TdR and harvested as above.

Typing for HLA-A, -B, and -DR antigens

A panel of alloantisera directed against the HLA-A and -B specificities as well as 50 antisera detecting HLA-DR antigens were used to type lymphocyte populations using the standard NIH microcytotoxicity and 8th Histocompatibility Workshop "B cell typing" microcytotoxicity assays.

Indirect immunofluorescence and flow cytometry

Lymphocytes (1.0 × 10^6) were washed and resuspended in RPMI containing 5% fetal calf serum, and incubated with 5 µl of OKIa antibody, a mouse monoclonal IgG2 directed against a monomorphic antigen present on all human HLA-DR molecules; from Ortho Pharmaceuticals, Raritan, New Jersey; (Reinherz et al. 1979). These were incubated at 4°C for 30 min, washed and reincubated with fluoresceinated goat antimouse IgG (monoclonal antibodies, Palo Alto, CA). Fluorescent labeled and appropriate control cell preparations were analyzed by fluorescence microscopy and flow cytometry (FACS IV). All fluorescence histograms and respective total energy values are standardized for 10^4 cells.

Blind technique

In all experiments, lymphocyte preparations were coded and then given to separate individuals for "blind" analysis of HLA phenotype, fluorescent microscopy, or flow cytometry.

Results

Abrogation of mitogen response, MLC response, and MLC stimulation by hyperthermia

Table 1 demonstrates that fresh human lymphocytes treated at 45°C are no longer able to activate allogeneic cells in a mixed lymphocyte culture. Responses stimulated by allogeneic heated cells are no greater than the background stimulation observed with irradiated autologous lymphocytes as stimulators. Furthermore, heated lymphocytes are unable to respond to alloantigens presented by irradiated allogeneic lymphocytes, and are unable to respond to PHA in a three-day assay. The effects of hyperthermia are dose dependent since treatment at temperatures less than 45°C diminishes, but does not totally abrogate the MLC and PHA responsiveness and the ability to stimulate in MLC. Similarly, treatment at 45°C for shorter periods of time decreases rather than eliminates the stimulation and responsiveness in MLC (data not shown). Even though these lymphocytes are functionally dead (no mitogen or MLC response or stimulation) following treatment at 45°C for 1 h, they still maintain some degree of membrane integrity based on their ability to exclude eosin as shown in Figure 1 (Roper & Drewinko 1976). In other experiments, heated or irradiated B and T cell enriched populations also showed comparable eosin exclusion kinetics as heated and irradiated unfractionated lymphocytes (data not shown).
Table 1. *In vitro* hyperthermia abrogates mitogen response, MLC response and MLC stimulation.

<table>
<thead>
<tr>
<th>Hyperthermia for 1 h</th>
<th>PHA response ((3^H)-Tdr cpm ((\times 10^3)))</th>
<th>MLC response ((3^H)-Tdr cpm ((\times 10^3)))</th>
<th>MLC stimulation ((3^H)-Tdr cpm ((\times 10^3)))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A_H + \text{PHA}^a)</td>
<td>(B_H + \text{PHA}^b)</td>
<td>(A_H + B_X^c)</td>
</tr>
<tr>
<td>Room temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43.0</td>
<td>150.8±22.4</td>
<td>68.0±8.1</td>
<td>36.4±5.2</td>
</tr>
<tr>
<td>44.0</td>
<td>77.3±2.5</td>
<td>39.3±1.8</td>
<td>18.5±2.6</td>
</tr>
<tr>
<td>44.5</td>
<td>20.0±2.8</td>
<td>2.5±0.4</td>
<td>18.8±8.9</td>
</tr>
<tr>
<td>45.0</td>
<td>0.32±0.04</td>
<td>0.36±0.23</td>
<td>7.6±1.0</td>
</tr>
<tr>
<td></td>
<td>0.27±0.13</td>
<td>0.37±1.9</td>
<td>0.18±0.12</td>
</tr>
</tbody>
</table>

Fresh human PBL were incubated at 43.0, 44.0, 44.5 and 45.0°C for 1 h and tested for their ability to respond to PHA (3 day assay), to respond to irradiated allogeneic cells in MLC (6 day assay) and stimulate allogeneic cells in MLC (6 day assay). All data are mean \(3^H\)-Tdr cpm ± S.E.M. of \(10^8\) responding cells in quadruplicate 0.25 ml microwells. The subscript "H" (i.e. \(A_H\)) indicates lymphocytes that have been treated with hyperthermia, while the subscript "x" indicates lymphocytes treated with x-irradiation (see Methods).

a. \(A + \text{media (3 day) control} = 0.14 ± 0.07\).
b. \(B + \text{media (3 day) control} = 0.33 ± 0.23\).
c. \(A + \text{media (6 day) control} = 0.50 ± 0.19\).
d. \(B + \text{media (6 day) control} = 0.26 ± 0.21\).
e. \(A + A_X^e\) positive control.
f. \(A + B_H^f\) positive control.
g. Additional controls in this experiment demonstrated that the addition of allogeneic 45.0°C heated cells did not abrogate the MLC response to allogeneic irradiated cells: \(B + A_H + A_X = 33.3 ± 8.4 \times 10^3\) cpm and \(A + B_H + B_X = 24.8 ± 4.4 \times 10^3\) cpm.

![Figure 1. Recovery of dye excluding heated or irradiated lymphocytes after culture. PBL (1.0 \times 10^8) from donors A and B were heated (\(A_H, B_H\)), irradiated (\(A_X, B_X\)), or untreated, and cultured in 0.20 ml HS-RPMI and incubated at 37°C. At the designated times, these lymphocytes were harvested, resuspended in 0.5% eosin, and counted in a hemacytometer. Cells able to exclude eosin were enumerated (mean of triplicate wells) and expressed as a percent of dye excluding cells obtained from the untreated population which had been incubated for the same time as the experimental populations. Seven-day recovery from these untreated populations was A = 0.68 \times 10^5, B = 0.70 \times 10^5.](image)
Hyperthermia does not diminish the fraction of PBL that express HLA-DR

The inability of heated cells to activate allogeneic cells in MLC suggests that the HLA-D antigens are functionally absent. Nevertheless, Table 2 demonstrates that heated lymphocytes contain nearly the same fraction of HLA-DR bearing cells as irradiated lymphocytes. In each experiment, responding lymphocytes (R) were stimulated with autologous irradiated lymphocytes (Rx) or irradiated unrelated stimulating cells (Sx). In addition, nylon wool adherent (B enriched) cells were irradiated (Sbx) or heated (Sbh). The heated cells did not stimulate in MLC, even though immunofluorescence with OK1a antibody showed minimal diminution of HLA-DR antigen bearing cells. Thus heat treatment did not eliminate HLA-DR antigens from the population, nor did it dramatically decrease the fraction of cells bearing HLA-DR antigens.

The specificity of HLA-DR antigens on heated cells

Despite the detection of the “monomorphic” portion of the HLA-DR antigen on heated cells by the OK1a antisera, it seems possible that heat treatment of human lymphocytes could alter this molecule in such a way as to eliminate its alloantigenic nature. This would account for the inability of heated cells to activate in MLC. We have ruled out this possibility by the detection of HLA-DR antigenic specificities on heated cells with alloantisera. We have found no appreciable difference in either the specificity of HLA-DR antigens recognized on heated lymphocytes, or the ability of these heated lymphocytes to be lysed by alloantisera plus complement. For example, untreated or heated B enriched lymphocyte preparations (the same used as stimulating cells in Experiment 1, Table 2) were tested with 50 separate B cell alloantisera in the standard HLA-DR microcytotoxicity assay. Both preparations were independently typed as DR1, DR4, MB1, MT3. Furthermore, as part of routine typing, the fraction of lysed cells was given a quantitative cytotoxicity

Table 2.
Detection of HLA-D, HLA-DR antigen on nylon wool enriched human B lymphocytes.

<table>
<thead>
<tr>
<th></th>
<th>HLA-DR antigen</th>
<th>HLA-D antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MLC response of donor R to test cell (3H-TdR cpm)</td>
<td>Percent of OK1a bearing cells</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rx</td>
<td>1,290</td>
<td>n.t.</td>
</tr>
<tr>
<td>Sx</td>
<td>49,130</td>
<td>4</td>
</tr>
<tr>
<td>Sbx</td>
<td>47,154</td>
<td>72</td>
</tr>
<tr>
<td>Sbh</td>
<td>1,118</td>
<td>53</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rx</td>
<td>626</td>
<td>n.t.</td>
</tr>
<tr>
<td>Sx</td>
<td>48,898</td>
<td>10</td>
</tr>
<tr>
<td>Sbx</td>
<td>28,363</td>
<td>74</td>
</tr>
<tr>
<td>Sbh</td>
<td>875</td>
<td>70</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rx</td>
<td>594</td>
<td>n.t.</td>
</tr>
<tr>
<td>Sx</td>
<td>23,605</td>
<td>8</td>
</tr>
<tr>
<td>Sbx</td>
<td>23,920</td>
<td>52</td>
</tr>
<tr>
<td>Sbh</td>
<td>648</td>
<td>46</td>
</tr>
</tbody>
</table>

PBL from donor S were irradiated (Sx) or enriched for B cells by nylon wool adherence and then irradiated (Sbx) or heated (Sbh). These were used to stimulate PBL from unrelated donor R in MLC, and were examined by microscopic indirect immunofluorescence for the percentage of OK1a bearing cells. Experiments 1, 2 and 3 are identical experiments each repeated on different days with different PBL donors.

a. MLC responses of 10⁵ responding lymphocytes measured after 6 days in vitro; 3H-TdR cpm of quadruplicates.

b. Percent of OK1a bearing cells detected by indirect immunofluorescence (fluorescent microscopy).

n.t. = not tested.
score from 1 to 8 for each serum tested (1 = 0–20% eosin positive, 8 = 80–100% eosin positive). Of the 50 antisera, 44 sera induced comparable cytotoxicity on the fresh irradiated B cells as on the heated B cells. There were six discrepancies, with four antisera showing greater cytotoxicity on the heated cells, and two sera showing greater cytotoxicity on the fresh cells. This serologic analysis demonstrates that the qualitative specificity of the HLA-DR antigen, as detected by alloantisera, is not appreciably altered by heating.

**Flow cytometry analysis of HLA-DR expression**

Unfractionated peripheral blood lymphocytes were irradiated or heated, and used as stimulators in MLC or labeled with OKIa antisera followed by FITC treated goat anti-mouse antisera. Figure 2-I shows that the number of fluorescent cells and the distribution of fluorescence intensity is virtually identical for the untreated and irradiated cells. Figure 2-II shows that the distribution of fluorescence intensity for the heated cells is

![Flow Cytometry Diagram](attachment:flow_cytometry.png)

Figure 2. Flow cytometry analysis of HLA-DR expression of heated and irradiated lymphocytes. Lymphocytes from donor A or B were irradiated (A_x, B_x), heated (A_H, B_H) or left untreated (A, B). These preparations were then labeled with OKIa antibody followed by FITC treated goat anti-mouse Ig and analyzed by flow cytometry. In each figure, two cell populations are compared for fluorescence. (See Methods for analysis specifications.)

2-I, A_x vs A; 2-II, A_H vs A, 2-III B_x vs B; 2-IV B_H vs B.

Quantitative fluorescent and MLC data corresponding to these same samples are presented in Table 3.
shifted slightly to the left from that of the untreated positive control cells. This demonstrates that there is less OKIa antisera detected per heated cell. The difference in fluorescence intensity is probably due to a minimal decrease in surface expression of the HLA-DR antigen on the heated cells; however, we cannot rule out "quenching" of fluorescence detection by heat treatment. Figures 2-III and 2-IV are comparable to 2-I and 2-II utilizing untreated, heated, and irradiated cells from lymphocyte donor B, and show that the fluorescence intensity of his heated cells with OKIa antibody is slightly less than that of untreated cells. Table 3 presents the MLC stimulating potential of these same cell preparations. As expected, heated cells are not stimulatory while comparable numbers of irradiated lymphocytes stimulate strong MLC responses. The flow cytometry analysis shows that the amount of HLA-DR on the heated (A_H or B_H) cells is approximately 75% the amount on the unheated cells. In other experiments (data not shown), the relative fluorescence intensity of lymphocytes treated at 45°C for 1 h, and unable to stimulate in MLC, ranged from 71.5 to 89.3% of control cells. Thus, 100 × 10^3 heated cells

Table 3. 
Hyperthermic abrogation of MLC does not correlate with a quantitative decrease in HLA-DR antigens added.

<table>
<thead>
<tr>
<th>Responder</th>
<th>Stimulator</th>
<th>Stimulating cells (×10^3)</th>
<th>^3H-TdRb (×10^3)</th>
<th>Relative fluorescence intensity</th>
<th>Percentc OKIa bearing cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A_x</td>
<td>100</td>
<td>8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>B_x</td>
<td>100</td>
<td>99.7</td>
<td>105.8</td>
<td>20.5</td>
</tr>
<tr>
<td>A</td>
<td>B_x</td>
<td>50</td>
<td>75.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>B_x</td>
<td>25</td>
<td>53.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>B_x</td>
<td>12.5</td>
<td>22.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>B_H</td>
<td>100</td>
<td>3.8</td>
<td>75.6</td>
<td>24.4</td>
</tr>
<tr>
<td>B</td>
<td>B_x</td>
<td>100</td>
<td>9.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>A_x</td>
<td>100</td>
<td>89.6</td>
<td>108.6</td>
<td>18.1</td>
</tr>
<tr>
<td>B</td>
<td>A_x</td>
<td>50</td>
<td>87.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>A_x</td>
<td>25</td>
<td>33.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>A_x</td>
<td>12.5</td>
<td>39.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>A_H</td>
<td>100</td>
<td>3.3</td>
<td>78.3</td>
<td>27.1</td>
</tr>
</tbody>
</table>

PBL from unrelated donors A and B were irradiated (A_x, B_x) or heated (A_H, B_H) and tested as allogeneic stimulating cells in MLC. These same heated and irradiated cell preparations were evaluated by FACS IV following labeling with OKIa antibody and FITC conjugated goat anti-mouse IgG.

a MLC data are ^3H-TdR cpm after 6 days of culture with 10^5 responding lymphocytes and the indicated number of stimulating lymphocytes.

b The relative fluorescence intensity was calculated from the relative fluorescent energy. This energy value (arbitrary units) was determined by multiplying the average cell frequency in each FACS window by the window number (1–255). The energy obtained with fresh cells was designated 100%, and the energy obtained with irradiated and heated cells are expressed as a percent of that control value. (Values obtained from numerical analysis of data presented in Figure 2.)

c The fluorescence obtained with fresh cells labeled only with the goat anti-mouse FITC reagent and no OKIa antibody was defined as background for this flow cytometry analysis. The designated populations, labeled with the OKIa antibody before the goat anti-mouse FITC, were analyzed by flow cytometry (Figure 2) for the percentage of PBL with more fluorescence per cell than 2 S.D. above background.
might be expected to bear roughly as much HLA-DR antigen as $75 \times 10^3$ irradiated cells. Nevertheless, as few as $12.5 \times 10^3$ irradiated cells still induce a detectable MLC response; in contrast, $100 \times 10^3$ heated lymphocytes are unable to activate in MLC. Thus the quantitative decrease in allogeneic HLA-DR antigen added to the culture (on the heated stimulating cells) is not a sufficient explanation for the total inability of heated cells to stimulate in MLC.

Discussion

Recognition of alloantigens by T cells appears to be as specific as recognition by alloantibody (Sondel & Bach 1975). However, cellular recognition is detected by a myriad of interacting cellular responses that are far more complex than binding of antigen by antibody. Interference with any of these steps, from initial antigen presentation to mitotic synthesis of the responding cells, may prevent detection of a proliferative immune response in vitro. We have shown here that heat treatment of either the stimulating or responding population of lymphocytes can totally abrogate the proliferative response in MLC. Because the multiple actions of hyperthermia on cell physiology include an antiproliferative (or lethal) effect, it is not surprising that adequate heating of PBL can abrogate their ability to respond immunologically (Bhuyan 1979).

In contrast, it remains unclear why heat treatment abrogates the ability to effectively present HLA-D alloantigens in the MLC reaction, while the ability to present HLA-A and -B antigens remains intact (Eijssenogel et al. 1973, Hank & Sondel 1982). In the present experiments we have shown that heated lymphocytes still bear their HLA-DR antigens, have cell membranes that exclude eosin for some time after heating, express unaltered DR alloantigens detected by alloantisera, and still express 75% of their surface DR molecules as estimated by flow cytometry.

These heated cells can still induce suppressor cells in an allogeneic MLC, although this suppression decreases, rather than abrogates, proliferative and cytotoxic responses to alloantigens (Sondel et al. 1977). Furthermore, when PBL are simultaneously stimulated with heated allogeneic cells and soluble antigen, or heated allogeneic cells and irradiated allogeneic cells, an effective helper and cytotoxic response is induced (Hank & Sondel 1982). Thus activation of suppressor cells cannot alone account for the absence of $^3$H-TdR incorporation in MLC with allogeneic heated cells.

Another potential mechanism that can be discounted as the sole mechanism of MLC abrogation by heat is macrophage inactivation. Proliferative responses to alloantigens and soluble antigens require the in vitro presence of adherent cells (monocytes). Allogeneic irradiated lymphocytes (B and T cells) depleted of adherent cells can activate in MLC only if adherent cells from the responding cell donor are present (Alter & Bach 1970). Similarly, proliferative responses to soluble antigens require that monocytes from the responding cell donor be present. The PBL preparations used as responding cells in these studies contain adherent cells and can respond well to mitogens (Table 1) and soluble antigens (i.e. tetanus, PPD, candida [data not shown]). Even if the "antigen processing" monocytes from the stimulating population were inactivated by heat, this function should have been supplied by monocytes from the responding cell donor (Germain 1981, Bergschohl et al. 1978). Therefore, heating must abrogate MLC stimulation by some other means.

There are at least five separate explanations consistent with the data accumulated to this point, to account for the inability of heated
lymphocytes to stimulate in MLC despite their continued expression of HLA-DR antigens.

First, the alloantigens responsible for MLC stimulation (HLA-D) may be totally distinct from the HLA-DR molecule which is genetically controlled within the same region (Segall et al. 1980). If so, hyperthermia treatment may have eliminated or altered the stimulating potential of the HLA-D molecule without significantly changing the expression of the HLA-DR molecules. This would prevent MLC stimulation by HLA-D yet enable the DR molecule to be detected by antibody, as in these studies.

Second, the HLA-D and HLA-DR antigenic determinants may be expressed on the same cell surface molecule; however, different domains of this molecule may be differentially susceptible to hyperthermia-induced damage. If so, the present experiments would suggest that HLA-DR expression is minimally altered by heat treatment and HLA-D expression is dramatically affected such as to prohibit effective alloactivation.

Third, heat treatment may not have altered HLA-D antigen expression any more than it altered HLA-DR expression. Namely, adequate amounts of these antigens could still be expressed on the cell surface of the stimulating population. If so, then active stimulation in MLC may require a separate heat sensitive "contribution" by the stimulating cell population in addition to its ability to present the HLA-D antigen. Possibilities for this "contribution" include: a) presentation or production of a distinct molecular entity which is non-polymorphic yet required for allore cognition of HLA-D (Germain 1981); b) activation of cell surface proteins following allomorphic interaction; c) flexibility of the cell surface to allow for mobility of surface proteins required for allore cognition; or d) ability to generate intracellular signals that can induce a separate intercellular message to be sent to the responding lymphocyte population. Other possibilities also exist. If heated cells express both the HLA-DR and -D antigens yet are unable to stimulate in MLC, then this other necessary "contribution" of the stimulating population may be the site of hyperthermic induced nonstimulation in MLC.

Fourth, the flow cytometry analysis suggests that somewhat less HLA-DR is expressed on each DR positive stimulating cell after heat treatment. This could be the result of a decrease in DR antigenic density on the surface of each cell or to a decrease in effective surface area per cell following hyperthermia (Welsch et al. 1977, Lin et al. 1973). If HLA-DR and HLA-D antigenic density are each decreased by hyperthermia, it may be the density of HLA-D on each individual stimulating cell that determines its stimulatory ability. The density of HLA-D expressed on heat-treated cells could potentially be below a critical "threshold" required for activation in MLC.

Finally, it remains unclear whether HLA-D region controlled surface molecules other than DR may be recognized by the monoclonal antibody utilized here. If the OKIa antibody actually recognizes the gene products of several HLA-D region loci, then the slight decrease in fluorescence intensity on heated cells could potentially reflect the loss of a subset of D region molecules needed to stimulate in MLC. The residual (i.e. approximately 75%) expression of "Ia" would then indicate the persistence of other D region molecules that would be unable to stimulate a primary MLC response. This may be clarified by an MLC analysis of HLA-D variant lines (Kavathas et al. 1981).

Which of these possibilities accounts for the hyperthermia induced non-stimulation remains undetermined. Hyperthermia is not the only cellular treatment that can decrease or abrogate MLC stimulating capacity. Excess mitomycin-C treatment, high dose gamma ir-
radiation, iodoacetic acid treatment or repeated freeze-thawing can accomplish this (Schellekens & Eijsvogel 1970). Comparable to our study is the demonstration that UV-treated human lymphocytes do not stimulate in MLC, despite their expression of HLA-DR (Slater et al. 1980). Thus, despite potentially different (and as yet unclear) mechanisms, hyperthermia and ultraviolet light treatment demonstrate a dichotomy between HLA-DR detection and the functional expression of HLA-D. Heated lymphocytes are intact enough to exclude eosin, do not suppressively abrogate other proliferative responses, and bear unaltered DR antigens, yet they cannot stimulate in MLC. Therefore the ability to present HLA-DR antigens is not sufficient to stimulate a primary MLC response.

Acknowledgments

We thank Drs. R. Hong and R. Auerbach for suggestions and critical discussions, and Ms. Grace Baumanns and Ms. Kathryn Edge for preparation of this manuscript.

References


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