Human/Mouse Radiation Chimera Are Capable of Mounting a Human Primary Humoral Response

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Lubin et al recently described a new approach that enables the generation of human/mouse chimera by adoptive transfer of human peripheral blood mononuclear cells (PBMC) into lethally irradiated normal strains of mice, radioprotected with bone marrow (BM) from donors with severe combined immune deficiency (SCID). In the present study, we demonstrate in such human/mouse chimera a marked humoral response to recall antigens, such as tetanus toxoid (TT) or hepatitis B surface antigen (HBsAg), as well as a significant primary response to keyhole limpet hemocyanin (KLH). Maximal anti-KLH response in human/Balb chimera was attained 2 to 4 weeks after the immunization and declined thereafter. One week after transplantation, the predominant anti-KLH subtype was IgM, while after 2 weeks, the dominance had shifted to IgG. Similar primary antibody response was also demonstrated against the human immunodeficiency virus (HIV) Nef protein. Comparison between human/Balb and human/SCID chimera showed a major difference in their ability to mount a primary response against KLH. In Balb/c recipients, more than half of the mice exhibited marked IgM titers against KLH, while there was hardly any anti-KLH IgM response in the SCID recipients. From the earliest time point onwards, when anti-KLH antibodies were found in the latter chimera, they were predominantly of the IgG type. We have previously shown that in human/Balb chimera, unlike in SCID recipients, dissemination of transplanted PBMC into the spleen and other internal organs occurs within 24 hours. Therefore, it is likely that the early seeding in the appropriate microenvironment of the lymphoid tissues, is crucial for the maintenance of virgin human B cells.

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THE OBSERVATION of Mosier et al that human PBMC can be adoptively transferred intraperitoneally (IP) into the SCID mouse and that the engrafted cells survive for an extended period of time producing high levels of human Ig, has offered many new possibilities in clinical immunology research. However, a major drawback of this approach, which deserves further improvements, is the relatively impaired functionality of the engrafted human lymphocytes. In particular, the T lymphocytes appear to be in a state of anergy, possibly because of imperfect antigen presentation in the xeno-environment of the mouse. Also, while the response to a recall antigen is very impressive, primary responses are minuscule or nonexisting.

Very recently Lubin et al described a new approach that enables the adoptive transfer of human peripheral blood mononuclear cells (PBMC) into lethally irradiated normal strains of mice radioprotected with SCID bone marrow (BM). It was demonstrated that, by this approach, it is possible to engraft human PBMC, not only to a variety of mouse strains including transgenic mice expressing human HLA surface antigen (HBsAg), as well as their primary response to keyhole limpet hemocyanin (KLH). We have previously shown that in human/Balb chimera, unlike in SCID recipients, dissemination of transplanted PBMC into the spleen and other internal organs occurs within 24 hours. Therefore, it is likely that the early seeding in the appropriate microenvironment of the lymphoid tissues, is crucial for the maintenance of virgin human B cells.

MATERIALS AND METHODS

Mice. Animals used were 6 to 10 weeks old. Balb/c and C58SCID mice were obtained from Olac Farms (Bicester, UK), and C3H/HeJ mice from Roscoe B. Jackson Memorial Laboratory (Bar Harbor, ME). All mice were kept in small cages (five animals in each cage) and fed sterile food and acid water containing ciprofloxacin (20 μg/mL).

Conditioning regimen. Balb/c mice were exposed to split dose (4 Gy followed 3 days later by 10 Gy) total body irradiation (TBI), from a gamma beam 150-A 60Co source (produced by the Atomic Energy of Canada, Kanata, Ontario) with FSD of 75 cm and a dose rate of 0.7 Gy/minute. C3H/HeJ mice were exposed to TBI of 4 Gy followed 3 days later by 11.5 Gy.

Preparation and transplantation of T-cell-depleted BM cells. BM cells obtained from SCID mice (4 to 8 weeks old) were fractionated by differential agglutination with soybean agglutinin (to remove T lymphocytes that may be present in occasional weakly SCID mice) according to Reisner et al with minor modifications. Recipient mice were injected intravenously (IV) with 2 to 3 × 10^6 SCID BM cells in 0.2 mL phosphate-buffered saline (PBS) 1 day after irradiation.

Preparation and transplantation of human PBMC. Buffy coats were depleted of red blood cells (RBC) by lysis in ammonium chloride solution (0.83% w/v). Eighty million PBMC were incubated in 1% Ringer solution at 37°C for 2 hours. The cell suspension was centrifuged at 300 × g for 10 minutes, washed twice in PBS, and finally resuspended in 2 mL of RPMI 1640. 1.0×10^7 cells were injected under the renal capsule of the recipient SCID or C58SCID mouse.

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from normal volunteers were layered onto Lymphoprep solution (Nycomed, Oslo, Norway) and spun at 2,000 rpm for 20 minutes. The interlayer was collected, washed twice, counted, and resuspended in PBS pH 7.4, to the desired cell concentration. Human PBMC (70 × 10^6 cells in 0.5 mL PBS) were injected IP into recipient mice, conditioned as described above. Control mice did not receive human PBMC.

**Leukapheresis procedure.** Leukapheresis was performed on normal volunteers or on volunteers who recovered from hepatitis B virus (HBV) infection and conferred protective immunity by generating high levels of antihepatitis B surface antigen (HBsAg) antibody. Cells were collected by processing 3 L of blood through a Baxter blood cell separator model CS 3000 (Deerfield, IL) during 1 to 1.5 hours. In the case of the normal volunteers, cells were collected by processing 3 to 4 L of blood through Haemonetics V50 (Braintree, MA) during 3 to 3.5 hours. The leukapheresis product was centrifuged at 1,200 rpm for 10 minutes and the plasma removed.

**Cells antigen/antibody collection from human/mouse chimera.** Animals were bled from the retro-orbital vein using heparin-coated glass capillaries. Plasma was kept for human Ig determination. Spleens were cut into small pieces and pressed through stainless steel sieves to make a cell suspension in PBS, and cells were then isolated using Lymphoperp.

**Antigens.** Tetanus toxoid (TT) for immunization was a commercial TT vaccine from RAFA Laboratories Ltd (Jerusalem, Israel). For anti-TT determination, a purified preparation of TT (kindly provided by RAFA Laboratories Ltd) was used. KLH was from Calbiochem-Behring Corp (La Jolla, CA). Nef was obtained through the AIDS Research and Reference Reagent program, AIDS program, National Institute of Allergy and Infectious Diseases (Bethesda, MD). Commercial HBsAg (Engerix-B) was from SIB Biological (Rixensart, Belgium). HBV-X antigen and a peptide of the HBV Pre-S1 (amino acids 21-47) conjugated to bovine serum albumin (BSA) were a generous gift from Dr Yosi Shaul (Virology Department, The Weizmann Institute of Science, Rehovot, Israel).

**Immunization of chimeric animals.** From a few hours to 3 days after PBMC transplantation, human/mouse chimera were immunized IP with different antigens. As adjuvant, we used either incomplete Freund adjuvant (IFA) (DIFCO Laboratories, Detriot, MI) emulsion, or aluminum hydroxide adjuvant (alum), prepared from aluminum potassium sulfate (Merck, D-6100 Darmstadt, Germany) as described. TT and HBsAg were injected IP with different antigens. As an adjuvant, we used either incomplete Freund adjuvant or aluminum hydroxide adjuvant (alum), prepared from aluminum potassium sulfate (Merck, D-6100 Darmstadt, Germany) as described. TT and HBsAg were injected IP with different antigens. As an adjuvant, we used either incomplete Freund adjuvant or aluminum hydroxide adjuvant (alum), prepared from aluminum potassium sulfate (Merck, D-6100 Darmstadt, Germany) as described.

**Human Ig determination.** Sera were tested for antigen-specific and total mouse Ig, as described above, by using goat-antimouse antibodies (Zymed Laboratories).

**Serum electrophoresis and immunofixation.** Electrophoresis and immunofixation serum samples were performed using commercial kits (Silenus Immunofixation Kit) from Silanes Laboratories Pty. (Hawthorn, Australia).

**Culture conditions.** All cell suspensions were cultured in RPMI medium supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL) (Bio-Lab, Jerusalem, Israel), 2 mmol/L L-Glutamine (Bio-Lab), 1% nonessential amino acid (Bio-Lab) and 10% heat inactivated bovine calf serum (HyClone, Logan, UT).

**In vitro B-cell activation.** Spleen cells (2.5 × 10^6 cells/mL, 1 mL) from human/mouse chimera were cultured in 24-well plates (Nunk, Denmark) in medium alone or in the presence of 2 μg/mL of pokeweed mitogen (PWM) (Sigma). Following 3 days of culture, the total amount of human Ig, as well as specific human anti-TT antibodies in the culture medium, were determined by ELISA as described above.

**Statistical analysis.** Statistical significance was established by the Student's t-test.

## RESULTS

**Characterization of human Ig subtypes in human/Balb chimera.** Lubin et al described the levels of human Ig in the sera of the human/Balb chimera at different time intervals posttransplant. In general, they found that maximal levels were reached at 2 to 3 weeks posttransplant and that these levels continued for at least 8 more weeks. We have now characterized, by ELISA, the level of the different human Ig subtypes in the sera of human/Balb chimera. Human IgG was found to be the dominant subclass in the chimeric mice (>90%), although both IgM and IgA could also be detected (Fig 1). Immunofixation of γ, μ, and α heavy chains also showed IgG dominance but, more importantly, demonstrated the polyclonality of the human Ig in these human/Balb chimeras. The polyclonality was also indicated by the presence of both light chains κ and λ (data not shown). ELISA for IgG subtypes showed the presence of all subtypes with a dominance of IgG1, >IgG2, >IgG3, >IgG4, (data not shown).

When spleen cells from human/Balb chimera were collected 2 weeks after transplantation and activated in vitro by PWM, marked levels of human Ig (217.3 ± 52.6 ng/10^6 spleen cells) could be detected in the culture medium. The antibodies were mainly of IgG subclass but, in some of the spleen cultures, high levels of IgM or IgA were found (data not shown). Lower (112.5 ± 12.9 ng/10^6 spleen cells), but significant, levels of human Ig were also found in the medium when the spleen cells were cultured in the absence of PWM.
Specific antibody response to a recall antigen. To evaluate the capacity of human/mouse chimera to generate human memory responses, human/Balb chimera were initially immunized, on the day of PBMC transplantation, with TT (1% lime flocculation [LF] in 0.2 mL). As shown by the typical experiment in Fig 2A, maximal levels of specific human anti-TT antibodies (8.3 ± 0.9 U/mL) were reached 4 weeks after the immunization. Significant levels continued to be detected for at least 4 more weeks. In some mice, human anti-TT was detected up to 12 weeks posttransplantation. A second immunization with TT did not enhance this human anti-TT response (data not shown).
The role of immunization in achieving this memory response was evaluated by comparing the anti-TT antibody level in TT immunized and nonimmunized human/Balb chimeras, the latter receiving irradiation alone without the antigen or a different antigen (KLH) with the same adjuvant. As shown in Fig 2A, a marked difference (P = .0001) was found between the groups.

The anti-TT response was predominantly of IgG type, but in some mice, IgM was also present (Fig 2B). IgA anti-TT levels were negligible. A test for murine anti-TT antibodies did not show any response of residual mouse lymphocytes that may have survived the lethal irradiation (data not shown).

The fraction of the anti-TT antibodies out of the total human Ig in the serum of human/Balb chimera immunized with TT was found to be enriched compared with the levels found in the sera of the original PBMC donors. Thus, 4 and 6 weeks posttransplant, the calculated mean enrichment factors were 23.2 ± 3.1 and 25.7 ± 3.3, respectively (Fig 2C).

Stimulation with PWM of spleen cells collected from human/Balb chimera 2 weeks after immunization with TT, led to marked production in vitro of specific human anti-TT (199.9 ± 68.5 × 10^5 u/10^7 spleen cells), which was predominantly of the IgG type.

Essentially, similar human anti-TT memory responses were obtained following immunization with TT of human/C3H chimera (data not shown). The response of chimeric mice to a recall antigen was also evaluated against HBsAg. In this study, C3H/HeJ mice were transplanted with human PBMC from a donor who recovered from acute HBV infection and seroconverted (anti-HBsAg positive). PBMC recipients were challenged with HBsAg (5 μg in 0.25 mL) within 24 hours of transplantation. In a typical experiment (Fig 3), the infused PBMC were collected by leukapheresis from a donor whose initial anti-HBsAg antibody serum level was 3,300 mU/mL. Two to 4 weeks after immunization of the human/C3H chimera, the serum anti-HBsAg level was greatly elevated (average of 32,460 ± 6,570 and 14,734 ± 6,442 mU/mL, respectively). Similar to the results with TT, a marked and significant difference was found between immunized and nonimmunized mice (Fig 3). Likewise, a marked enrichment (up to an enrichment factor of 423) for specific anti-HBs antibodies, was found in the serum of immunized chimeric mice compared with the original serum level of the donor. When C3H mice were transplanted with PBMC from the same donor and challenged with the HBV-X antigen or with a peptide of pre-S1 (conjugated to BSA), instead of HBsAg, the level of anti-HBs antibodies was less than 10% of the response observed in the mice immunized with HBsAg, similar to the spontaneous background level exhibited by nonimmunized human/C3H chimera (Fig 3). When C3H mice were transplanted with PBMC from anti-HBsAg negative donors and challenged with HBsAg, no specific anti-HBs response could be detected (Fig 3). The correlation between the level of anti-HBs titers in the donor serum and the antibody titers achieved in the human/mouse chimera, was further substantiated when PBMC from sero-positive donors with different titers of anti-HBs antibodies were used to generate human/mouse chimera (Table 1). Thus, although transplantation of PBMC from donors no. 1 or no. 2, whose anti-HBsAg levels were low (650 and 928 mU/mL, respectively) resulted in human/mouse chimera with a substantial serum level of total human Ig, these chimera exhibited an extremely poor response (35 ± 18.7 and 201 ± 126.3 mU/mL, respectively) while in recipients of human PBMC from donors with relatively high original serum levels (donors 3 to 6) the average anti-HBsAg level in the recipients from each of these donors was above 11,000 mU/mL.

**Specific primary antibody response.** To investigate the capacity of human/mouse chimera to mount a primary response, we tested initially their response to immunization with KLH. Human/Balb chimera were immunized 1 to 3 days posttransplantation with KLH (50 μg in 0.2 mL of

### Table 1. Serum Level of Human Anti-HBsAg Ig (mU/mL) in PBMC Donors and in Human/Mouse Chimeras 2 Weeks After Immunization With HBsAg

<table>
<thead>
<tr>
<th>Donor</th>
<th>Original Donor</th>
<th>Human/Mouse</th>
<th>No.</th>
<th>Total Human Ig in Human/Mouse Chimeras (μg/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>650</td>
<td>35 ± 18.7</td>
<td>6</td>
<td>4,992 ± 625</td>
</tr>
<tr>
<td>2</td>
<td>928</td>
<td>201 ± 126.3</td>
<td>14</td>
<td>5,448 ± 970</td>
</tr>
<tr>
<td>3</td>
<td>3,300</td>
<td>32,460 ± 5,570</td>
<td>23</td>
<td>4,948 ± 453</td>
</tr>
<tr>
<td>4</td>
<td>5,447</td>
<td>33,621 ± 3,374.2</td>
<td>34</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>4,700</td>
<td>11,242 ± 6,448</td>
<td>11</td>
<td>2,774 ± 324</td>
</tr>
<tr>
<td>6</td>
<td>7,790</td>
<td>20,763 ± 10,733</td>
<td>5</td>
<td>3,404 ± 637</td>
</tr>
</tbody>
</table>

Human/mouse chimera transplanted with 70 × 10^6 human PBMC. Immunization IP with 5 μg HBsAg at the day of transplantation.

Abbreviation: ND, not determined.

* Average ± SE.
IFA). Sera were obtained from recipient mice weekly, and assayed by ELISA for specific anti-KLH human Ig.

Initially, four groups of chimeric mice transplanted with cells from four different donors were tested, and individual positive mice from all donors responding to KLH were found in every group. Maximal anti-KLH average response was attained 2 to 4 weeks after the immunization and declined thereafter (Fig 4A). A second immunization with KLH did not enhance this human anti-KLH response (data not shown).

To determine the role of KLH immunization in generating the anti-KLH response, the response of human/Balb chimera immunized with KLH was compared with that of chimera immunized with TT. As can be seen in Fig 4B, a highly significant \( P = 0.0002 \) difference between the two groups was observed, while no difference was found between the two groups in their total human Ig level. No difference in anti-KLH response was found when alum was used for immunization as the adjuvant instead of IFA (data not shown).

The relative dominance of different Ig subclasses in the specific anti-KLH average response is shown in Fig 4C. The pattern of the anti-KLH response during the first weeks following immunization in two individual mice is illustrated in Fig 4D. One week after transplantation, the predominant anti-KLH subtype was IgM, while after 2 more weeks, the dominance had shifted to IgG. The IgA levels were insignificant.

Human primary humoral responses were also found to be generated in human/C3H chimera against the HIV protein Nef (50 µg in 0.2 mL of IFA). When tested by ELISA at a titer of 1:800, 60% (12 of 20) of the immunized chimera exhibited human anti-Nef IgM and IgG antibodies.

Comparison of anti-KLH response in human/Balb versus human/SCID chimera. The observation that a primary response against KLH could be generated in human/Balb chimera is in marked contrast to several studies, which concluded that following infusion of human PBMC into SCID mice, the ability of the engrafted cells to mount a primary response was minuscule or completely diminished. Therefore, to establish whether a significant difference exists between the two models, an identical number of cells from the same individuals (collected by leukapheresis) were infused into large groups of recipient SCID and conditioned
Balb/c mice, and the respective primary response against KLH of the resultant chimera was compared at weekly intervals.

The results of two independent experiments using two individual donors showed a major difference in the magnitude and in the quality of the response between the human/Balb and the human/SCID chimera. As can be seen in Figs 5A and B, the total human anti-KLH Ig, measured by ELISA at 1:200 serum dilution, was significantly higher in the human/Balb chimera.

Analysis of human anti-KLH IgM (Fig 6A, b from one donor and Fig 6A, d from the second) and human anti-KLH IgG (Fig 6A, a and Fig 6A, c, respectively) in the human/mouse chimera, showed a marked qualitative difference between the SCID and the Balb/c recipients. In the SCID recipients who were able to respond to KLH, this response was predominantly limited to the IgG class, while IgM responses were negligible. On the other hand, in the Balb recipients the IgM response was dominant, beginning at week 1 and reaching a maximal level 3 weeks after transplantation. This marked IgM response was accompanied in the Balb/c recipients with a gradual increase in anti-KLH IgG responses (Fig 6A). The difference in the IgM response between human/Balb and human/SCID chimera was highly significant ($P = .0001$) throughout the follow-up period, while the small difference in IgG response was significant only during the first 2 weeks after immunization ($P = .0007$).

Determination of serum anti-KLH titers at 2 weeks post-transplant showed that altogether, in both experiments, 14 of 18 engrafted Balb/c recipients exhibited anti-KLH IgM response at 1:3,200 serum dilution, whereas among the SCID recipients, only three of 35 engrafted mice exhibited anti-KLH IgM response at 1:800 serum dilution (Fig 6B).
DISCUSSION

The initial observation of Mosier et al.\textsuperscript{18} that human PBMC can be adoptively transferred into SCID mice has led to numerous studies using this model for different purposes, including HIV infection,\textsuperscript{19} human autoimmunity\textsuperscript{20,21} and outgrowth of human lymphocytes from cancer patients.\textsuperscript{22,23} In particular, the capacity of engrafted lymphocytes to generate humoral responses has been studied in great detail,\textsuperscript{2} and it was conclusively shown that, while a significant response can be induced to a recall antigen such as TT\textsuperscript{24,25} or GP160 (after immunization of normal volunteers and subsequent engraftment of PBMC from such individuals in SCID mice),\textsuperscript{26} HBs,\textsuperscript{27} erythrocyte D-antigen\textsuperscript{10} or isoagglutinins,\textsuperscript{28} primary responses were completely negative (against KLH,\textsuperscript{2} extremely low (against \(\alpha_{174}\)) or in low frequency of mice (against HBs\textsuperscript{3} or HB core antigen\textsuperscript{a}). In one study only in which human/SCID chimera prepared according to Mosier were immunized with schistosome antigen, was a significant primary-like response documented.\textsuperscript{13} However, no IgM titers were shown to illustrate that, indeed, a primary response had taken place.

Our present results demonstrate that following adaptive transfer of human PBMC into lethally irradiated normal strains of mice, prepared according to Lubin et al.,\textsuperscript{14} humoral responses to recall antigens such as TT or HBs,\textsuperscript{2} as well as primary responses to KLH and Nef, can be generated effectively.

The distinction between a memory response and a primary response to a naive antigen is not straightforward, as most naive antigens could be cross-reactive to some extent with other recall antigens. Indeed, we and others (A. Donenberg, unpublished results) were able to detect, when using sufficiently sensitive ELISA, low IgM anti-KLH titers in the serum of most unimmunized PBMC donors, whereas antibodies against NEF were less common (data not shown). Thus, it could be argued that the anti-KLH IgM titers found in the human/mouse chimera may represent a superposition of a truly naive response together with some expansion and secretion of IgM by previously sensitized cross-reactive IgM producing B cells. Therefore, the primary response in our model can only be defined operationally, with all the interpretation limitations regarding the stage of differentiation of the B cells eliciting the observed responses. Typically, this response is associated with an initial dominance of the IgM sub-type, while the memory response toward recall antigens, against which the PBMC donor has been previously vaccinated, is dominated by the IgG sub-type.

Although the literature regarding the feasibility of primary responses in the Mosier model almost uniformly rules out the possibility of generating a significant primary response against KLH in this model, it was of interest to evaluate the primary IgM response attained in the Balb/c recipients in comparison to that achieved in SCID mice, by using the same donors and by infusing the same number of cells. The results of these experiments clearly showed a major difference in the capacity of the engrafted cells to mount an IgM response against KLH. Thus, while in Balb/c recipients more than half of the mice exhibit marked IgM titers against KLH, there is hardly any anti-KLH IgM response in the SCID recipients. Anti-KLH antibodies in the latter chimera are predominantly of the IgG type, probably as a result of a recall response, reflecting the potential cross-reactivity of KLH epitopes with other antigens. Altogether, these results clearly show the superiority of the new model for the adoptive transfer of such operationally defined primary responses in comparison to the standard SCID/human chimeric system.

The lack of primary humoral response in our human/mouse chimera after immunization with HBsAg is not surprising considering the weak humoral responses elicited by commercial vaccines in normal individuals with fully competent immune systems. Thus, the anti-HBsAg titers in normal immunized individuals are minuscule compared with the titers found in seropositive patients (data not shown). Likewise, adoptive transfer of PBMC from immunized donors into lethally irradiated mice leads to negligible serum titers compared with chimeric mice transplanted with PBMC from seropositive donors (data not shown). Two independent sets of observations suggest a possible correlation between early dissemination of human lymphocytes into lymphoid organs and the capacity of engrafted mice to mount a primary human response against KLH.

In SCID recipients, dissemination of engrafted human lymphocytes into the spleen or lymph nodes is a slow process, requiring weeks,\textsuperscript{2} and the majority of infused cells remain at the site of administration in the peritoneum cavity. As shown above primary responses against KLH are very weak in such chimera. In contrast, very recently Sandhu et al.\textsuperscript{25} found that rapid dissemination of human PBMC could be achieved in SCID recipients upon extreme conditioning of the mice with 3 Gy TBI plus treatment with antiasialo-GM1. While this treatment is lethal, due to enhanced graft-versus-host disease (GVHD) and the marked radiosensitivity of SCID mice, the recipients survive 3 to 4 weeks during which time a primary anti-KLH IgM response can be elicited. These investigators suggested that, although KLH responders become sick and eventually die by the fourth week posttransplant, KLH-specific B cells can be potentially recovered for immortalization from such chimera.

The major difference found in the present study between human/Balb and human/SCID chimera in their capacity to mount an IgM response against KLH, may also result from differential engraftment of naive human B cells, the conditions for which may not be optimal in SCID mice.

Rapid dissemination of engrafted human cells was previously demonstrated\textsuperscript{13} in the radiation chimera, shown in our present study to mount a human IgM response against KLH. Moreover, Burakov\textsuperscript{a} et al found that human B lymphocytes (positive for CD20 by immunohistology) arrive in the white pulp of the spleen and form primary follicular centers in the spleen and in the lymph nodes (unpublished results). Therefore, it is likely that the early seeding in the appropriate microenvironment of the lymphoid tissues is crucial for the maintenance of virgin human B cells, which may not survive in the peritoneum cavity. It is possible that this site can only support the propagation of human memory B cells and fails...
to maintain naive cells. However, at present, we cannot rule out the possibility that the heavy irradiation of the recipient mice could enable selective engraftment of other human lymphocyte subpopulation or antigen presenting cells critical for generation of primary antibody responses.

Clearly, the advantages associated with rapid engraftment of functional PBMC, in the absence of significant lethal GVHD or complications due to the emergence of EBV lymphoma (which commonly leads to monoclonal dominance in human/SCID chimera as early as the fifth week posttransplantation) could now be afforded by the human/Balb chimera. Thus, such human/mouse chimera may be useful, not only for the generation of sensitized human B cells as fusion partners for monoclonal antibody production, but also by virtue of their survival for the provision of a viable model for testing the efficiency of new vaccines or immunopotentiating drugs.

However, it should be noted that both primary and memory responses were achieved in the chimeric mice upon presentation of the antigen immediately after infusion of the human PBMC. Administration of the antigen later than 1 week after transplantation, led to poor results (data not shown). Likewise, several attempts to boost the response by a secondary challenge with the antigen, were not effective. This shortcoming of the model could be explained by the previous observation of Lubin et al. that human CD14+ cells can be detected by fluorescence-activated cell sorter (FACS) only during the first week after the transplant, indicating that effective antigen presentation can, perhaps, be achieved in the chimeric mice only during this early critical period. Antigen presentation in the context of the appropriate donor type HLA, may be improved by using CD14+ antigen presenting cells (from the original donor) primed with the specific antigen for subsequent challenges.

In summary, although further studies are required to fully explore the functionality of engrafted human PBMC in normal mice or rats, the ability to induce primary and memory responses in immunocompetent recipients of different genetic backgrounds, while maintaining the engrafted mice, will enable, on the one hand, generation of antigen specific human B cells for immortalization and, on the other hand, will allow evaluation of new vaccines, as well as new approaches, including different boosting modalities or the use of transgenic mice carrying human genes (such as the histocompatibility class-II antigens or human cytokines), for further improvement of antigen presentation, isotype switching, and other important functional aspects of engrafted human cells.

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