ANTIBODY TO THE MURINE TYPE 3 COMPLEMENT RECEPTOR INHIBITS T LYMPHOCYTE-DEPENDENT RECRUITMENT OF MYELOMONOCYTIC CELLS IN VIVO

BY HUGH ROSEN, GENEVIEVE MILON, AND SIAMON GORDON

From the Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, United Kingdom

The delayed-type hypersensitivity (DTH) skin response elicited in immunized hosts reflects discrete, sequential events in different anatomical compartments (1-3). After immunization, antigen-specific T cells are activated and clonally expanded in draining lymphoid tissues. Activated antigen-specific T cells recirculating in the blood are mobilized to the skin if the antigen (ag) is reinjected. Once restimulated by accessory cells, T cells secrete a range of interleukins, including IFN-γ and IL-2 that promote local recruitment of myelomonocytic cells from blood and alter the phenotype of surrounding endothelial cells, fibroblasts and keratinocytes (4-7). Induction of a DTH response may be either protective (7, 8) or deleterious (9) depending on the final balance between eradication of an infective organism and damage to host tissues.

The type 3 complement receptor (CR3) plays and important role in leukocyte adhesion and recruitment to an immunologically nonspecific inflammatory stimulus (10). The CR3 belongs to the leukocyte function-associated 1 (LFA-1) family of surface receptors (11) and forms part of the integrin superfamily of cell adhesion molecules (12) which mediate a wide range of cell-cell and cell-substratum interactions (13). Human patients deficient in the LFA-1 family of molecules, namely LFA-1, CR3, and p150/95, display defective leukocyte adhesion and a decreased ability to recruit myelomonocytic cells to extravascular sites (14, 15), but since all three molecules are absent or reduced on the surface of leukocytes, it is not possible to ascribe distinct functions to individual LFA-1 family members. A murine model in which CR3 function is perturbed by the use of a specific mAb, 5C6 (10), has allowed the assessment of the contribution of CR3 alone during inflammatory responses within the intact animal. In this paper we have examined the extent to which T cell-dependent inflammation induced by specific ag challenge in sensitized mice, or by adoptive transfer in naive, syngeneic mice is inhibitible by an mAb (5C6) directed specifically to CR3.

This work was supported by the Medical Research Council and the Arthritis and Rheumatism Research Council. H. Rosen is a Junior Research Fellow of Jesus College, Oxford. G. Milon was on leave from the Institut Pasteur, Paris, France and was the recipient of a NATO fellowship. Address correspondence to H. Rosen, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom.

1 Abbreviations used in this paper: Ag, antigen; CR3, type 3 complement receptor; DTH, delayed-type hypersensitivity; LAD, leukocyte adhesion deficiency; LFA, leukocyte function-associated; Mø, macrophage.
Materials and Methods

Mice. 8-wk-old C57/BL6 female mice were obtained either from the Sir William Dunn School of Pathology, Oxford or the specific pathogen-free unit of the Institut Pasteur, Paris, France.

Immunization and Treatment of Mice. SRBC in Alsevers solution (Gibco Laboratories, Paisley, Scotland) were washed three times in Dulbecco's A PBS and resuspended in sterile normal saline. 10⁶ SRBC were injected intravenously into the tail vein. Mice were immunized with 3 × 10⁶ viable BCG organisms (Institut Pasteur) (16) by subcutaneous injection in both hind footpads.

Assay of the DTH to SRBC. 4 d after immunization mice were tested for DTH by an antigenic challenge of 10⁴ or 10⁵ SRBC injected subcutaneously in a volume of 50 μl into the right hind footpad. Footpad swelling was measured at a range of times with a dial gauge caliper and the measurement of the uninjectected footpad was subtracted from that of the challenged footpad. Footpads were subsequently removed and fixed in formal saline. The wax-embedded footpads were then sectioned and stained with hematoxylin and eosin. The local adoptive transfer of the DTH reaction in syngeneic naive mice was performed as described (17). Briefly, 4 d after immunization, mice received 50 IU of heparin. Heparinized blood was collected 15 min later, diluted and used as a source of SRBC-sensitized T cells. This was then mixed with either SRBC or unrelated ag and injected into one hind footpad of naive, syngeneic recipients and the increase in footpad thickness was examined 14-18 h later. Footpads were processed for histological examination as above.

Assay of the Adoptively Transferred Inflammatory Response to Tuberculin. 4-6 d after BCG immunization of the footpads, draining lymph nodes were removed, teased apart, and the single cell suspension was used as a source of tuberculin-reactive T lymphocytes. 1.5 × 10⁶ cells were mixed with 50 μg of tuberculin and injected in a volume of 1 ml into the peritoneal cavity of naive, syngeneic mice (18). Peritoneal cells were harvested 48 h later, counted, and cytospin preparations were then examined immunocytochemically by an indirect immunoperoxidase technique (19) to allow an accurate assessment of the different cells recovered. Specifically, the proportion of macrophages (Mφ) was assessed by staining cytospin preparations for the F4/80 ag (20) with a polyclonal rabbit antiserum prepared against purified murine F4/80 ag by Dr. Pietro Dri in this laboratory. This permitted the specific staining of Mφ even when labeled with the 5C6 rat mAb.

The Effect of mAb 5C6 on Expression of DTH. 5C6, a rat IgG2b mAb directed against the murine CR3, was prepared and purified as described (10). 1 mg of IgG was injected intravenously either at the time of ag challenge or up to 6 h later and the effects were compared with controls in which either buffer alone or a control rat IgG2a mAb 7/4 (21) restricted to murine myelomonocytic cells, and with a similar site number to 5C6 or the F(ab')² fragment of 5C6 that has no in vivo activity (10) were injected. In experiments where local injection of sensitized T cells and ag was used to adoptively transfer DTH, 5C6, or a control preparation was injected intravenously to donor mice at the time of immunization to assess possible effects on the development of ag-reactive T cells. Recipients were injected with mAbs intravenously at the time of local transfer to influence systemic delivery of inflammatory cells. Alternatively, the transferred blood cells were preincubated with mAb and then washed by centrifugation before local injection.

The Persistence of mAb after a Single Intravenous Injection. The adequacy of circulating 5C6 IgG was assessed in two ways. First, serial blood samples were taken at times from 1 h to 4 d after a single injection of 1 mg i.v. The serum was then diluted in Iscove's medium (Flow Laboratories, Paisley, Scotland) with 5% FCS and 4-d thiglycollate-elicited Mφ were then incubated in the diluted serum before measurement of adhesion to bacterial plastic as described (10). 4 d after injection, a 1:10 dilution of the sampled serum still inhibited adhesion to plastic by >85%. In addition, the degree of mAb binding to blood leukocytes, peritoneal cells, and bone marrow cells was assessed at 1, 4, 24, 48, and 72 h after a single intravenous injection of FITC-5C6, by fluorescence analysis on a FACS II apparatus (Becton Dickinson & Co., Mountain View, CA). Saturation binding to myelomonocytic cells alone was seen, and persisted for the duration of the experiment.
Assay of the Half-Life of 5C6 IgG and F(ab')2 in the Circulation. Mice were injected i.v. with 3,000 U of intact IgG or F(ab')2. 50 μl of blood were collected from the tail veins at 10 min, and at 2, 4, 8, and 24 h after injection. Serial dilutions of this blood were assayed for binding activity as described (10). Binding was defined in an indirect RIA to adherent glutaradehyde-fixed, thioglycollate-elicited macrophages. The reciprocal of the dilution of antibody that gives rise to 50% maximal binding of a fixed quantity of iodinated second antibody is designated the number of binding units per milliliter. In this assay, 1 mg/ml of pure 5C6 IgG contains 6,300 binding units.

Results

Effect of mAb 5C6 on DTH after Active Immunization with SRBC. There is convincing evidence that the footpad DTH measured in the mouse after intravenous immunization with a low dose of SRBC is a good model of T lymphocyte-mediated recruitment of myelomonocytic cells (22-24). The main features of this DTH are a maximal specific response 4 d after active immunization, which reaches a peak at ~20 h after ag challenge. This SRBC-specific response can be transferred systemically or locally to naive, syngeneic mice only by transfer of cell suspensions containing Thy-1+, CD4+ lymphocytes from SRBC-sensitized mice (24). The adoptive DTH response is not detectable when sensitized T lymphocytes are transferred to mice that have been lethally irradiated 30 h before transfer. In such recipients in which the bone marrow and blood myelomonocytic cell pool is depleted, the DTH response is restored only by the intravenous injection of bone marrow cells (24). We have therefore examined the importance of CR3-mediated myelomonocytic adhesion in this immunologically specific inflammatory response.

Fig. 1 shows the increase in footpad thickness seen after local ag challenge with SRBC 4 d after active systemic immunization. Naive animals (Fig. 1 A) showed...
a negligible increase in footpad thickness of 0.10 ± 0.05 mm 20 h after ag challenge compared with the significant increase of 1.26 ± 0.28 mm seen in the footpad size of the actively immunized control mice (B). The degree of increased footpad thickness was dependent upon the amount of ag injected as the high dose of SRBC (10⁸ cells/footpad, B) elicited 2.3 times the swelling seen with a 10-fold lower amount of ag (0.54 ± 0.16 mm, F). Intravenous injection of 1 mg of 5C6 mAb at the time of ag challenge completely abolished the footpad swelling seen at 20 h in both high (C) and low dose (G) ag challenges. Delay of the intravenous injection of 5C6 mAb for up to 6 h after ag challenge still led to highly efficient inhibition of the footpad swelling at 20 h (D). The specificity of the 5C6 IgG effect is shown in Table I where the inhibitory effect of 5C6 IgG on footpad swelling is compared with buffer alone, a control rat mAb 7/4 binding to circulating myelomonocytic cells with a similar site number 5C6 (21) or the pepsin F(ab')² fragment of 5C6, none of which inhibited footpad swelling. The half-life of injected 5C6 IgG and P(ab')² was found to be 7.5 and <2 h, respectively.

The ability of 5C6 to inhibit the DTH at 20 h was confirmed on histological examination of the footpad sections. Fig. 2 A shows the 20-h response to injected SRBC in a naive mouse. Intact SRBC are seen in the extravascular tissue spaces. The host response to these erythrocytes was minimal with an occasional infiltrating neutrophil or monocyte. In contrast, the actively sensitized mouse challenged with SRBC in the absence of 5C6 mAb shows an obvious inflammatory infiltrate at 20 h, composed of a mixture of monocytes and neutrophils (Fig. 2 B). In actively immunized mice injected intravenously with 5C6 IgG at the time or up to 6 h after ag challenge, intact SRBC are seen dispersed in the extravascular tissue space without any accompanying myelomonocytic inflammatory infiltrate (Fig. 2 C).

The CR3-dependent and CR3-independent Recruitment of Myelomonocytic Cells to DTH. Fig. 3 illustrates the time course of the footpad swelling seen after ag challenge of actively immunized mice in the presence or absence of 5C6. The control sensitized mice showed significant increases in footpad thickness within 12 h of ag challenge. This swelling then reached a peak at 24 h, subsided to 42% of the peak level at 48 h, and returned to the baseline at 72 h. Mice injected with 5C6 at the time of ag challenge had an increase in footpad size of 0.08 ± 0.07 mm at 24 h compared with 0.90 ± 0.17 mm of the sensitized controls. By 48 h, the 5C6-treated mice had an

### Table I

<table>
<thead>
<tr>
<th>Immunization i.v. (day 0)</th>
<th>Challenge s.c. (day 4) Ab i.v.</th>
<th>Increase in footpad thickness mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>SRBC</td>
<td>0.05 ± 0.05</td>
</tr>
<tr>
<td>SRBC</td>
<td>SRBC</td>
<td>1.05 ± 0.10</td>
</tr>
<tr>
<td>SRBC</td>
<td>SRBC</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>SRBC</td>
<td>SRBC</td>
<td>0.90 ± 0.20</td>
</tr>
<tr>
<td>SRBC</td>
<td>SRBC</td>
<td>1.05 ± 0.17</td>
</tr>
</tbody>
</table>

Assay as described in Materials and Methods. Antibodies (1 mg) were injected intravenously at the time of ag challenge. Results show the mean ± SD of four mice in each group from one representative experiment.
increase in footpad size of 0.39 ± 0.07 mm, which was not different to the declining increase of 0.38 ± 0.05 in the untreated sensitized mice. From 48 h onwards, the curves of the 5C6-treated and the control mice were virtually superimposable. A second injection of 5C6 20 h after ag challenge failed to alter appreciably the late...
ANTI-CR3 INHIBITS ANTIGEN-SPECIFIC INFLAMMATION

1.50

图3. 5C6注射对SRBC挑战后足垫厚度时间进程的影响。结果是每组每实验的四只敏感化小鼠的平均±SD。在三组独立实验中得到了类似的结果。

5C6注射后的足垫大小的增加在48 h时在5C6处理的小鼠中出现。与SRBC注射的小鼠并未在任何时候出现足垫大小的增加。

循环5C6 IgG水平在1 mg IgG单次注射后进行稀释分析。该单克隆抗体的抗粘附滴度通过细胞培养中骨髓中性粒细胞或诱发Mφ来检测（10），并通过饱和血液水平来维持，至少72 h。FACS分析表明5C6与血液、骨髓和腹腔细胞的结合在注射后1 h内达到饱和，并在72 h内保持稳定。在任何阶段都没有发现白细胞减少、细胞毒性或差别的血细胞计数的改变。骨髓标记在1 h时较弱，达到饱和后24 h，然后维持稳定直到实验结束。因此得出结论，循环中的5C6 IgG在功能和结合标准上都达到要求，且在实验中，不充分的mAb水平未被考虑。

5C6在时间进程中的效果再次在组织学检查中明显可见。在20 h时，5C6抑制了炎症细胞的招募（图2C）。与SRBC在组织空间中可见到的数目很少对应的细胞充满了红细胞碎片（图2E）。相反，5C6注射的小鼠在48 h时的炎性浸润是足够解释那时的肿胀（图2F）。大部分的SRBC仍然在血管外的组织空间中。

我们得出结论，招募到挑战的足垫的单核巨噬细胞的招募是是二相的。在激发的动物中，它由一个早期的CR3依赖的成分和一个晚期的非依赖CR3成分组成。在48 h时，SRBC在激发的动物中出现了明显的炎症浸润。在5C6注射的小鼠中，这种浸润在48 h时非常小，足够解释那时的肿胀。5C6保持了完整的SRBC在血管外的组织空间中。

我们得出结论，招募到挑战的足垫的单核巨噬细胞的招募是二相的。在激发的动物中，它由一个早期的CR3依赖的成分和一个晚期的非依赖CR3成分组成。在48 h时，SRBC在激发的动物中出现了明显的炎症浸润。在5C6注射的小鼠中，这种浸润在48 h时非常小，足够解释那时的肿胀。5C6保持了完整的SRBC在血管外的组织空间中。
phase that accounts for most of the footpad swelling and the bulk of inflammatory cell recruitment. There is a second CR3-independent phase that accounts for the modest swelling and cellular infiltration seen at 48 h, the time at which the increased footpad thickness in 5C6-treated and control mice becomes indistinguishable, although obvious differences in both cellular infiltration and ag persistence remain.

Local and Systemic Administration of 5C6 in Naive, Syngeneic Mice: Effects on Local Transfer of DTH. Although CR3 is restricted to the myelomonocytic lineage and is not present on resting or activated lymphocytes (10), it is important to demonstrate that the 5C6 mAb limits its effects to inhibition of myelomonocytic recruitment and does not impair the ability of T lymphocytes to become activated and to transfer DTH. To assess the effects of 5C6 on the development of functional sensitized T cells, mice were injected intravenously with 5C6 and then immunized with SRBC. 4 d later, sensitized T cells were harvested, washed by centrifugation, mixed with SRBC, and injected into the footpad of naive, syngeneic mice. Table II shows that the footpad swelling seen after passive transfer of ag and blood leukocytes was similar using donor leukocytes recovered from mice immunized with SRBC in the presence or absence of systemic 5C6.

We also examined the effect of 5C6 injection in recipient mice on hypersensitivity following passive local transfer of SRBC-reactive T cells and SRBC. Local transfer led to acute onset of DTH and reached a peak at 15 h after ag challenge. Table II shows that local transfer to naive, syngeneic recipients of sensitized blood leukocytes mixed with SRBC led to an increase in footpad size of 0.52 ± 0.05 mm in control mice at 15 h while leukocytes or ag alone failed to elicit any response.

| Table II |
| mAb 5C6 Inhibits T Cell-dependent Myelomonocytic Recruitment but not ag-dependent T Cell Priming or Activation |

<table>
<thead>
<tr>
<th>Treatment (donors)</th>
<th>Transfer to recipients</th>
<th>Increase footpad thickness (recipients) at 15 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Blood leukocytes</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>SRBC</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Blood leukocytes + SRBC</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>SRBC</td>
<td>Blood leukocytes</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>SRBC</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Blood leukocytes + SRBC</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Recipients treated 5C6 IgG i.v.</td>
<td>0.10 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Blood leukocytes + SRBC</td>
<td>0.58 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Transferred cell preincubated 5C6 and washed before transfer</td>
<td>0.65 ± 0.12</td>
</tr>
<tr>
<td>SRBC + 5C6 IgG</td>
<td>Blood leukocytes</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>SRBC</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Blood leukocytes + SRBC</td>
<td>0.65 ± 0.12</td>
</tr>
</tbody>
</table>

Performed as described in Materials and Methods. Results reflect the mean ± SD of four mice per group. Equivalent results were obtained in each of two separate experiments.
If mice were injected with 5C6 intravenously at the time of local transfer of sensitized cells and ag, increase in footpad size was significantly diminished at 0.10 ± 0.08 mm at 15 h. This result was confirmed by histologic observation (data not shown).

Effect of 5C6 on Adoptive Transfer of T Cell-dependent Inflammatory Reaction to Tuberculin. The effect of 5C6 was not restricted to inhibiting T cell-dependent recruitment of inflammatory cells to one ag alone at a single anatomical site. We have analyzed recruitment of monocytes to the peritoneal cavity after simultaneous injection of BCG-sensitized T lymphocytes and tuberculin. This system has the advantage of allowing sampling of peritoneal cells and accurate assessment of the number of inflammatory cells recovered. We chose to examine the cellular response at 2 d to highlight the CR3-dependent recruitment of monocytes.

Fig. 4 shows the effects of 5C6 on inflammatory recruitment after transfer of sensitized lymphocytes and tuberculin to the peritoneal cavity of naive, syngeneic mice. Naive mice not injected with cells or ag, or injected with either cells or ag alone, yielded 4.65 ± 1.68 × 10^6 peritoneal leukocytes of which 45% (2.1 × 10^6) were MΦ by virtue of their expression of the F4/80 ag by immunocytochemistry. 2% were neutrophils and 53% lymphocytes. 48 h after transfer of 1.5 × 10^7 BCG-sensitized lymph node cells and 50 μg tuberculin, the number of peritoneal cells had increased to 9.12 ± 2.29 × 10^6 cells, of which 66% (6.10 × 10^6) were MΦ, 8% neutrophils, and 26% lymphocytes. In contrast, mice treated by the intravenous injection of 5C6 at the time of transfer of sensitized cells and tuberculin had only 4.28 ± 2.22 × 10^6 peritoneal leukocytes at 48 h of which 47% (2.01 × 10^6) were MΦ, 3% neutrophils, and 50% lymphocytes. Injection of tuberculin alone failed to elicit any quantitative or qualitative changes in the recovered cell population. The cellular response to the local injection of BCG-sensitized T cells mixed with tuberculin was predominantly monocytic in nature and was completely inhibited by the intravenous injection of 5C6 mAb, if the initial resident population is taken into account.

Discussion

These studies in which we have inhibited the DTH in vivo using a CR3-specific mAb highlight a role for CR3 in T cell-dependent recruitment of myelomonocytic leukocytes. These data extend our observations that an mAb restricted to the CR3
can inhibit leukocyte responses to thioglycollate broth (10). There is no evidence in either of these experimental models that 5C6 IgG functions in vivo by cytotoxicity or the depletion of myelomonocytic cells from blood or bone marrow. Its inhibitory effects in vivo correlate thus far with its in vitro inhibition of CR3-dependent adhesion and spreading (10). We conclude that CR3 is important either directly or indirectly in the migration of myelomonocytic cells to inflammatory sites in both immunologically specific and nonspecific processes. The mechanism(s) by which the mAb achieves this inhibition of recruitment remain(s) unclear.

**5C6 Inhibition of the Early Phase of DTH.** 5C6 efficiently inhibits the first 24 h of the DTH to SRBC with respect to the parameters of footpad swelling and myelomonocytic cell recruitment on histological section. This effect is specific for the 5C6 IgG and is absent using the F(\(ab\)')\(_2\) pepsin fragment capable of blocking iC3b binding, but not nonspecific inflammation (10). The F(\(ab\)')\(_2\) fragment is rapidly cleared after injection with a half-life of \(<2\) h, unlike the IgG with its 7.5-h half-life. This rapid clearance is similar to that described for rabbit antibodies and fragments (25). Inhibition of recruitment with the F(\(ab\)')\(_2\) might require constant infusion to maintain adequate circulating levels. The myelomonocytic-restricted 7/4 mAb had no inhibitory effects on either footpad swelling or cellular infiltration.

After 24 h the inhibitory effects of 5C6 were less evident with gradual swelling of the footpad to \(~40\%\) of the peak swelling seen in the control group at 48 h and then returning to normal by 72 h. This swelling was accompanied by limited infiltration of both neutrophils and monocytes to the site of injection, though removal of SRBC was limited. Functional adhesion assays and monitoring of cell labeling using FACS showed that adequate levels of 5C6 were maintained in both intravascular and bone marrow leukocyte compartments throughout the duration of the experiments. The time course of the DTH response is therefore consistent with a biphasic process, a first CR3-dependent recruitment phase that peaks at 24 h and then subsides, while the second phase appears CR3-independent and rises after 24 h to reach a lower peak at 48 h.

A possible explanation for the failure of 5C6 to inhibit swelling at 48 h is that CR3 may not be the sole cell adhesion molecule that can mediate migration of myelomonocytic cells to a T cell-dependent inflammatory site. Local persistence of a particulate ag such as SRBC and the presence of additional recruitable sensitized T cells may lead to the prolonged production of T cell secretory products such as IFN-\(\gamma\), that with time may alter or induce expression of ligands on the adjacent endothelial cell surfaces for other leukocyte adhesion molecules. Candidate leukocyte receptors include LFA-1 (26), p150,95 (27), members of the VLA family (28), monocyte \(\Pi b-\Pi I a\) (29), and possibly MEL-14 (30), while molecules such as ICAM-1 (31) and ELAM-1 (32) are potential endothelial surface ligands. These possibilities should be examined in vitro and in vivo using recombinant cytokines and other mediators. Another possibility is that T cell products lead to damage to the endothelial monolayer and expose the basement membrane. Myelomonocytic cells with their well-documented abilities to bind substratum components such as fibronectin (33) and laminin should then be able to leave the vascular compartment. 5C6 does not impair the adhesion of \(\text{M}^\Phi\) or neutrophils to fibronectin (10).

Although in the present studies we found a marked difference in T cell-dependent myelomonocytic recruitment in mice treated with an anti-CR3 mAb, altered
DTH responses have not been deemed to be a prominent feature of leukocyte adhesion deficiency (LAD) in man. Anderson et al. (34) found a positive cutaneous hypersensitivity response to Candida albicans antigens in two of three patients with severe deficiency of the LFA-1 family glycoproteins. The remaining patient with severe deficiency was reported to have had a negative Candida skin test. Patients with moderate deficiencies tended to have positive responses to Candida and the one reported biopsy of a Candida test showed perivascular infiltration by mononuclear leukocytes similar to the stromal infiltration seen in a gingival biopsy from the same patient. The SRBC model, while being a good T cell-dependent model of inflammation, differs from the DTH in humans in a number of important respects. The cells recruited are myelomonocytic rather than mononuclear, the window during which sensitized T cells are circulating is narrow, and the response is characterized by rapid swelling and relatively rapid resolution without prolonged induration. Furthermore, comparison with the findings in the human patients is hampered by the absence of published data on the nature or extent of the infiltrate in the indurated areas of cutaneous hypersensitivity. It will be important to extend present studies to other DTH models in the mouse, and to investigate the time course and extent of the DTH response in the human LAD patients.

5C6 Inhibition of Local Transfer of DTH to SRBC in Naive, Syngeneic Mice. We used passive transfer of DTH to restrict the experiment to the examination of myelomonocytic cell recruitment alone. The failure of 5C6 injection in donor mice at the time of sensitization to have any effect on transfer of DTH to recipients, rules out the possibility that 5C6 acts by interfering with T cell sensitization. The inhibitory effect of systemically injected, but not of locally injected 5C6, reinforces the notion that 5C6 acts only at the level of myelomonocytic cells and not on lymphocyte migration or reactivation at the local site. 5C6 inhibition of the local transfer was complete and the second CR3-independent phase of recruitment and footpad swelling found in the actively immunized mice was not present. The passive transfer experiments are thus a simplification of the model.

5C6 Inhibition of Monocyte Recruitment to Locally Transferred Sensitized T Cells and Tuberculin. The inhibition of DTH to tuberculin provides important additional data. First, it shows that the role of CR3 is not restricted to specific recruitment of myelomonocytic cells to one ag alone. Second, unlike DTH to SRBC, our data from the DTH response to tuberculin in the peritoneal cavity show that it is predominantly monocytic rather than a neutrophil-rich mixture of myelomonocytic cells. This more closely resembles the DTH response in the human. Under these experimental conditions, as in the passive transfer of DTH to SRBC, 5C6 IgG causes almost complete inhibition of the DTH response even after 48 h with no CR3-independent phase. Treatment with 5C6 mAb also allowed the separation of the resident and recruited peritoneal leukocyte populations with only resident cell numbers being recovered after 5C6 treatment.

The present study implicates CR3 as a significant molecule in the T lymphocyte-dependent recruitment of myelomonocytic cells to an inflammatory site, while not elucidating the mechanism by which CR3 acts in these models. The precise role of CR3 could be revealed by mAbs that show functional inhibition in vivo as monovalent fragments, although the rapid clearance of such fragments might pose technical difficulties in performing such experiments. Such reagents are currently being sought.
This study extends previous observations by ourselves (10) and others using reagents directed against either the specific α chain of CR3 (35, 36) or the common LFA-1 β chain (37–39) that CR3 is critical in the genesis of nonspecific inflammatory responses. We have highlighted the potential of the CR3 as a target for antiinflammatory drug therapy likely to be useful in a range of clinical conditions characterized by a deleterious inflammatory process, irrespective of whether such inflammation is immunologically specific or not. There will, however, also be a range of T cell-dependent responses, including transplant rejection and the response to viral pathogens where the contribution of CR3 is of lesser importance (Rosen, H., and S. Gordon, unpublished observations). Impairment of CR3-dependent myelomonocytic recruitment in animals mounting a cell-mediated immune response to a rapidly proliferating organism such as Listeria monocytogenes can also be profoundly deleterious (Rosen, H., S. Gordon, and R. J. North, manuscript in preparation), indicating that the use of such a strategy to inhibit inflammation should be treated with caution.

Summary

We have used the delayed-type hypersensitivity (DTH) response to SRBC or tuberculin to examine the role of the murine type 3 complement receptor in T lymphocyte-dependent inflammatory recruitment. Intravenous injection of 5C6, a CR3-specific rat mAb known to impair myelomonocytic adhesion, divided the DTH to SRBC in actively immunized mice into two phases. The early phase, which lasted 24 h, was characterized by maximal oedema and maximal inflammatory recruitment and was 5C6 inhibitable. The later phase was 5C6 resistant and reached a peak 48 h after antigenic challenge and was superimposable on the declining peak seen in control mice. Passive transfer of reactive T cells mixed with antigen was used to examine the myelomonocytic effector arm of the DTH alone. Both passive transfer of cutaneous DTH to SRBC and passive transfer of the largely monocytic T cell-dependent recruitment to tuberculin in the peritoneal cavity were completely abolished by systemic 5C6 treatment. Injection of 5C6-treated donor leukocytes at the site of passive transfer had no effect. Treatment of donor mice with 5C6 at the time of active immunization did not alter their ability to provide reactive T cells for passive transfer. The myelomonocyte-restricted rat mAb 7/4 and the rapidly cleared F(ab')2 fragment of 5C6 showed no inhibition of the DTH. In all cases, inhibition of footpad swelling correlated with histological evidence of inhibition of myelomonocytic cell recruitment. Peritoneal cell counts after local DTH to tuberculin showed complete inhibition of monocyte recruitment. We conclude that CR3 plays a quantitatively important role in T cell-dependent inflammatory recruitment. This is absolute in passive transfer experiments, but only partial after active immunization. Leukocyte CR3 plays a common role in both immunologically specific and nonspecific inflammatory recruitment and provides a target that could possibly be manipulated to therapeutic advantage.

We thank Mr. J. Kent for skilled assistance with animals, Drs. Gheorghiu for BCG, F. Romain for tuberculin, Peter Tree for mAb 7/4, Les Haven for histology, and Stan Buckingham and Cathy Lee for photography.

Received for publication 20 June 1988 and in revised form 3 October 1988.
ANTI-CR3 INHIBITS ANTIGEN-SPECIFIC INFLAMMATION

References


