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B Cell Complement Receptor 2 Transfer Reaction

Margaret A. Lindorfer,* Hasmig B. Jinivizian,*, Patricia L. Foley, † Adam D. Kennedy,*, Michael D. Solga,* and Ronald P. Taylor2,*

The B cell C receptor specific for C3dg (CR2) shares a number of features with the primate E C receptor (CR1). Previously, we have demonstrated, both in vitro and in animal models, that immune complexes (IC) bound to primate E CR1, either via C opsonization or by means of bispecific mAb complexes, can be transferred to acceptor macrophages in a process that also removes CR1 from the E. We have now extended this paradigm, the transfer reaction, to include B cell CR2. We used both flow cytometry and fluorescence microscopy to demonstrate that IC bound to Raji cell CR2, either via C opsonization or through the use of an anti-CR2 mAb, are transferred to acceptor THP-1 cells. This reaction, which appears to require Fc recognition of IgG bound to Raji cell CR2, also leads to transfer of CR2. Additional support for the B cell transfer reaction is provided in a prototype study in a monkey model in which IC bound to B cell CR2 are localized to the spleen. These findings may have important implications with respect to defining the role of C in IC handling during the normal immune response.

Complement serves a major effector function in the adaptive and innate immune systems as well as in facilitating production of a robust immune response (1–4). Ags reacted with Abs to form immune complexes (IC)3 are more immunogenic than free Ags, and opsonization of IC by C to generate C3dg-labeled IC is critical in enhancing immunogenicity. In fact, interaction of opsonized IC with C receptor 2 (CR2), the C3dg receptor on B cells and follicular dendritic cells (FDC), plays a key role in the proliferation and maturation of the immune response (5–11). Our laboratory has focused on the role of the primate E C receptor 1 (CR1) in IC processing (12). We have demonstrated that IC bound to E CR1 via either C3b- or C-independent bispecific mAb constructs are transferred to acceptor phagocytes in a reaction in which FcR recognition leads to removal of CR1 from E and uptake of the entire IC and CR1 by the acceptor cell (13, 14).

B cell CR2 shares structural and functional features with E CR1 (15). The extracellular domains of both receptors are composed of multiple copies of the short consensus repeat (SCR), a folding motif containing 60 aa, several of which are invariant. The ligand for E CR1 is C3b; the ligand for CR2 is the downstream degradation product C3dg (9, 11, 15, 16). Under many conditions, CR1 and CR2 levels on B lymphocytes as well as CR1 on leukocytes and E are reduced (17–21); in fact, soluble CR2 is found in the circulation in normal individuals (22–24), and at increased levels in certain diseases (23, 24). CR2 can be shed and/or reduced in copy number on B or T cells, and one or more unidentified proteases may cut CR2 (22–24). Based on the intriguing similarities in the properties of CR1 and CR2 both in humans and in mouse models (25–27), we investigated the ability of IC bound to B cell CR2 to participate in a transfer reaction analogous to that observed with E CR1. Our findings may have important implications with respect to the role of C3dg-IC in the normal processing of Ags during the immune response.

Materials and Methods

Human E and cell lines

The human monocytic THP-1 cell line (American Type Culture Collection (ATCC), Manassas, VA) was cultured in 10% FBS, in RPMI 1640 medium (ATCC) supplemented with gentamicin (Life Technologies, Grand Island, NY). THP-1 cells were treated with retinoic acid (Sigma-Aldrich, St. Louis, MO) 3–4 days before use (28), and except for the experiment in Fig. 1, G and H, were dyed with the fluorescent dye PKH26 (Sigma-Aldrich), according to the manufacturer’s directions. The B lymphoblastoid Raji cell line (ATCC) was cultured in the same medium supplemented with penicillin and streptomycin (Life Technologies). Cultured cells were pelleted from growth medium (200 × g), resuspended in standard medium (5%, 10% FBS in RPMI 1640 medium (Life Technologies)), and held at room temperature until opsonized or dyed with PKH26. Whole blood from normal donors was anticoagulated with EDTA and stored in 50% Alsevers.

Antibodies

Anti-human CR2 IgG2a mAb HB135 (29) and bacteriophage ßX174 (30, 31) were labeled with Alexa (Al) 488, 594, or 633 (Molecular Probes, Eugene, OR), according to the manufacturer’s directions. Anti-ßX174 mAbs 51.1, 7B7, and 7A4 have been previously described (30). Mouse mAb IH8 was produced by immunizing mice with C3b(i)-Sepharose (32); binding of this mAb to C3b(i)-Sepharose was blocked by purified C3d (Advanced Research Technology, San Diego, CA). Combinations of unlabeled mAb HB135 (intact or its Fab’2 fragments), Al488 mAb HB135, Al488 rabbit anti-mouse IgG (Molecular Probes), FITC anti-CD21 (clone BL13; Beckman Coulter Immunotech, Marseille, France), and allophycocyanin (APhCy) anti-CD21 (clone B-ly4; BD PharMingen, San Diego, CA) were used to probe cells after transfer. The anti-CR2 mAbs HB135, BL13, and B-ly4 recognize distinct and non-overlapping epitopes on Raji cells, and on monkey B cells (only HB135 and B-ly4 tested in monkeys; data not shown). The Fab’1 fragment of mAb HB135 was prepared with the ImmunoPure kit, according to the manufacturer’s directions (Pierce, Rockford, IL). Anti-CR1 mAb IB4 (33) and anti-CR2 mAb PE8 (34) were used to block binding of C-opsonized IC to E or Raji cells, respectively. FITC mAb F10-89-4 (mouse IgG2a; Serotec, Raleigh, NC) and APhCy mAb H130 (mouse IgG1; Caltag, Burlingame, CA), both specific for human CD45, were used to opsonize Raji cells and probe for loss of CD45 after transfer, respectively.

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Cell opsonization and transfer reaction protocols

To bind ΦX174 to human E CR1 via immune adherence, washed E (10% hematocrit) were incubated in 50% normal human serum (NHS)/50% SM with A1488 ΦX174 (15 μg/ml) and three anti-ΦX174 mAbs (2 μg/ml each) for 7 min at 37°C. After three washes, E were resuspended in RPMI 1640 medium (Life Technologies) and combined at varying ratios (3:1 to 20:1) with THP-1 cells in RPMI 1640 medium. Aliquots of the mixtures were centrifuged at 200 × g for 5 s and incubated at 37°C for varying times. The transfer reaction for each aliquot was stopped by dilution into ice-cold BSA-PBS, followed by centrifugation and resuspension of the cell pellet in 1% paraformaldehyde in PBS (1% PF-PBS).

The details of Raji cell transfer experiments and fluorescent probing schemes are summarized in Table I. To bind ΦX174 to Raji cell CR2 via C, A1488 ΦX174 (10 μg/ml) and a mixture of two or three anti-ΦX174 mAbs (2 μg/ml each mAb; comparable binding was obtained for both cocktails) were incubated in 50% NHS/50% SM for 15 min at 37°C to deposit C3dg on the IC. An equal volume containing 5 × 10^7 cells/ml in SM with 2 mg/ml goat IgG was then added to the serum-treated IC, and the mixture was incubated for an additional 15 min at 37°C. After three washes, the Raji cells were combined at varying ratios (1:3 to 1:1) with THP-1 cells, and the transfer reaction was conducted as described for E. After transfer, washed cells were resuspended, blocked, and probed (Table I), and then washed twice with BSA-PBS and resuspended in 1% PF-PBS. We used similar conditions to examine direct binding of ΦX147/anti-ΦX174 IC to THP-1 cells in either medium, NHS, or NHS-EDTA.

Alternatively, Raji cells (2.5 × 10^7 cells) were ligated with anti-CR2 mAb (12.5 μg mAb HB135 or A1488 HB135, or 8 μg Flb(H)), HB135 for 30 min at 37°C, washed three times with BSA-PBS, and, for some experiments, incubated with 6.25 μg A1488 rabbit anti-mouse IgG for an additional 30 min at 37°C (see Table I). The Raji cells were washed three times with BSA-PBS, resuspended at 1 × 10^7 cells/ml in RPMI 1640 medium, and combined at varying ratios (1:3 to 1:1) with THP-1 cells. Control studies in which CR45 on Raji cells was targeted used a similar approach, based on binding of FITC mAb F10-89-4. Preliminary binding tests with unlabeled anti-CR45 mAb F10-89-4 or anti-CR2 HB1135, followed by development with A1488 anti-mouse IgG revealed that Raji cells express levels of CD45 comparable to CD21, and that CD45 is also well expressed with a BX40 fluorescence microscope (Olympus, Melville, NY), equipped with a Magnafire digital camera (Olympus), using FITC, FITC/Texas Red (FITC/TR), or TR filters.

Results

Binding and transfer of C-opsonized IC

We have previously described binding of C-opsonized IC to private E CR1 and their transfer to acceptor cells (13). We have also demonstrated that Ags can be bound to E in the complete absence of C by use of a bispecific complex, an anti-CR1 mAb chemically cross-linked to an anti-Ag mAb. These E-bound IC are transferred to acceptor cells in a concerted reaction in which CR1 is removed from the E (12, 14, 30, 35). We first examined C-dependent binding of ΦX174/anti-ΦX174 IC to E CR1 and Raji cell CR2 under various conditions (Table II). Binding of IC to either cell type with a BX40 fluorescence microscope (Olympus, Melville, NY), equipped with a Magnafire digital camera (Olympus), using FITC, FITC/Texas Red (FITC/TR), or TR filters.

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required NHS, and if C activity was abrogated by heat inactivation or addition of EDTA, no binding was detected. If E or Raji cells were pretreated with mAbs specific for the ligand binding site on CR1 or CR2, respectively, IC binding was eliminated. Experiments with mAb 1H8, specific for C3dg, confirmed that this C fragment was associated with Raji cells with bound C-opsonized IC. Treatment of these cells with mAb FE8 led to release of both the bound IC and C3dg (data not shown).

For purposes of comparison, we investigated transfer of C-opsonized IC from E to THP-1 cells. X174 was labeled with Al488 to allow direct detection of IC, and THP-1 cells were labeled with PKH26 to distinguish them from donor cells. C-opsonized IC-opsonized IC, bound to E CR1 are stable in medium for 60 min (Fig. 1A, ○). However, when combined with THP-1 cells, E-bound IC were rapidly released and transferred to THP-1 cells (Fig. 1, A and B, ●).

This transfer reaction was also followed by fluorescence microscopy. Fig. 1C shows the red fluorescence from a PKH26-dyed THP-1 cell observed with the FITC/TR filter before incubation with E. After 30 min of transfer, a red acceptor THP-1 cell is identified in Fig. 1E, and Fig. 1, D and F, displays the green fluorescent IC (containing Al488 X174 initially bound to E) associated with this cell. Fig. 1G shows green fluorescent IC bound to E before transfer to undyed THP-1 cells; Fig. 1H illustrates that after a 60-min incubation, most of the green fluorescent IC are indeed associated with THP-1 cells.

Similar experiments were performed with Raji cells as donor cells. When the Al488 X174/anti-X174 IC was incubated in NHS for 30 min at 37°C, stable binding of Al488 X174 to Raji cells was detectable by flow cytometry (Fig. 2A, ○). However, in the presence of THP-1 cells, the IC were rapidly transferred (Fig. 2, A and B, ●). We probed the cell mixtures for CR2 and found, as previously observed by others (24), that Raji cells display a slow rate of spontaneous loss of CR2 when incubated alone (Fig. 2C, ○ and △). Coincubation with THP-1 cells greatly increased the rate of loss of CR2 from Raji cells containing bound IC, but not from
naive Raji cells (Fig. 2C, compared with Fig. 2E).

Both the intrinsically labeled Al488 IC (Fig. 2B) and CR2, as indicated by the extrinsic anti-CR2 probe (Fig. 2D), were transferred to THP-1 cells. In contrast to the behavior of the intrinsic Al488 IC label, the extrinsic signal rose rapidly and then slowly declined to 70% of its peak value (Fig. 2D). Thus, both IC and CR2 were removed from the donor cell and transferred to the acceptor cell with very similar kinetics. The subsequent decrease in magnitude of extrinsic signal attributable to CR2 on THP-1 cells may reflect either internalization by or release from the THP-1 cells.

We also followed transfer of bound IC from Raji cells to THP-1 cells by fluorescence microscopy. The top panels of Fig. 2E show a red fluorescent PKH26-dyed THP-1 cell and a Raji cell opsonized with green fluorescent IC immediately after mixing. The bottom panels illustrate transfer of green IC from Raji cells to THP-1 cells after 30 min.

Binding and transfer of C-independent anti-CR2 mAb-based complexes

To extend this work to a C-independent paradigm, we opsonized Raji cells by ligation with the anti-CR2 mAb HB135 (29). As shown in Fig. 3, both CR2 and the anti-CR2 mAb were transferred from Raji cells to THP-1 cells; the intrinsic and extrinsic probes demonstrated similar kinetics of transfer to THP-1 cells. To more closely simulate a B cell-bound IC in the absence of C, Raji cells previously incubated with unlabeled mAb HB135 were further reacted with Al488 rabbit anti-mouse IgG (Fig. 4). The intrinsic Al488 label (rabbit anti-mouse IgG; Fig. 4, C and D) was transferred at a rate similar to that observed for the transfer of mAb HB135 (Fig. 3, A and B). After transfer, cell mixtures were probed with Al633 goat anti-rabbit IgG (Fig. 4, A and B) or APHcy anti-CR2 (Fig. 4, E and F). As seen for IC prepared with C (Fig. 2, C and D), the extrinsic probes showed an initial rapid rise in the THP-1-associated signal, followed by a decrease over the next 20 min. When Al488 HB135 and unlabeled rabbit anti-mouse IgG were used to opsonize Raji cells, similar kinetics of transfer of the intrinsic and extrinsic labels was observed (data not shown).

In view of the similarities in structure and function of CR1 and CR2, we thought it likely that Raji cell CR2 should be processed in the transfer reaction in a similar fashion to the processing of E CR1. In contrast, it would seem reasonable that structurally unrelated cell surface proteins would not be transferred after engagement by specific mAbs and exposure to THP-1 cells. To address this question, it is necessary to use isotype-matched mAbs to allow for testing in the paradigms illustrated in Fig. 3. Based on these considerations, we have examined the fate of CD45 (36) on Raji cells when it is bound by IgG2a mAb F10-89-4 and then incubated with THP-1 cells (Fig. 5). Although small amounts of the anti-CD45 mAb are lost from the Raji cells during the experiment,
development with a noncompeting second mAb specific for CD45 indicates no loss of CD45 from the Raji cells under conditions that do lead to removal of CD21, when it is bound by IgG2a mAb HB135. Moreover, this targeted loss of CD45 during the transfer reaction does not lead to loss of CD45 (data not shown).

To determine whether the Fc portion of IgG was necessary for the transfer reaction, we opsonized Raji cells with the F(ab'){	extsubscript{2}} fragment of mAb HB135 and compared its transfer with that of cells reacted with intact mAb HB135 (Fig. 3). As demonstrated previously (Fig. 3), intact mAb HB135 and associated CR2 were simultaneously transferred from the Raji cell to the acceptor cell. However, for cells opsonized with the F(ab'){	extsubscript{2}} fragment of mAb HB135, neither the mAb fragment nor CR2 was removed from the donor cell or taken up by THP-1 cells. Thus, the Fc portion of the substrate appears to be necessary for transfer. To further examine the possible role of FcR in the transfer reaction, we preincubated THP-1 cells with human IgG. Human IgG in the reaction mixture blocked transfer of an anti-CR2 mAb/rabbit anti-mouse IgG IC from Raji cells (Fig. 6). Loss of both A1488 rabbit anti-mouse IgG and CR2 from the Raji cells was reduced (Fig. 6, A and C, □), and the rate of loss was similar to the rates observed in the absence of THP-1 cells (data not shown). Moreover, in the presence of human IgG, THP-1 cells did not take up either A1488 rabbit anti-mouse IgG or CR2 (Fig. 6, B and D, □).

In view of the apparent role of FcR in the transfer reaction, it is possible that binding of IgG, contained in IC, to FcR on the THP-1 cells might activate these cells and lead to nonspecific proteolysis of Raji cell CR2. We examined the conditions that optimize binding of F(ab'){	extsubscript{2}} fragment to THP-1 cells. Robust binding, presumably mediated by FcR, occurs when IC are added to THP-1 cells, but binding is reduced in serum, and is eliminated in serum-EDTA (Table III). In analogy to the findings in Fig. 6, we suggest that human IgG in serum and serum-EDTA blocks IC binding by competing for binding sites on FcR. The lower, but discernible level of binding obtained in serum is most likely caused by C activation, followed by C3b-mediated immune adherence of the opsonized IC to CR1 (37). Therefore, in subsequent experiments, THP-1 cells were pretreated with preformed IC in the absence of serum. To test for nonspecific proteolysis of CR2, naive Raji cells were incubated alone, with naive THP-1 cells, or with THP-1 cells containing bound IC. The results (Fig. 7) indicate that the modest loss of CR2 from naive Raji cells alone (∆) is not increased by incubation with THP-1 cells, with or without bound IC (△, ○). However, as illustrated previously above, CR2 is removed and transferred from the Raji cells when IgG2a mAb HB135 is first bound to the cells, and they are then reacted with THP-1 cells; binding of IC to the THP-1 cells substantially inhibits this reaction, presumably because of FcR blockade. In a similar experiment, Raji cells were opsonized with A1488 HB135. Pretreatment of the THP-1 cells with IC reduced the loss of A1488 HB135 on the Raji cells from 62 ± 2% to 35 ± 1%, relative to naive THP-1 cells. Finally, transfer of NHS-opsonized, A1488-labeled IC from Raji cells to THP-1 cells is also inhibited when IC are prebound to the THP-1 cells. The fluorescent signal of Raji cells opsonized with A1488 F(ab'){	extsubscript{2}}-anti-CR2 IC in NHS decreased from 540 ± 4 to 292 ± 8 (channel number) when naive THP-1 cells were acceptors, vs 540 ± 4 to 408 ± 3 when THP-1 cells were pretreated with IC, corresponding to a reduction in transfer from 90 to 70%, respectively.

**Primate study**

In vitro calibration studies with whole blood from four cynomolgus monkeys established that the anti-human CR2 mAb HB135 (but not an isotype control) recognizes CR2 on monkey B cells; in

**FIGURE 6.** Transfer does not occur if the substrate lacks an Fc region, or if FcR on the THP-1 cell are blocked. After 30 min, 77% of mAb HB135 and 72% of CR2 were removed from Raji cells ligated with mAb HB135, compared with 27% loss of mAb and 11% loss of CR2 from Raji cells with HB135 F(ab'){	extsubscript{2}}. Representative of two similar experiments. After 30 min, 55% of the IC and 61% of CR2 were removed from Raji cells incubated with THP-1 cells, while only 14% of the IC and 17% of CR2 were removed from Raji cells incubated with THP-1 cells in the presence of 1 mg/ml human IgG.

**FIGURE 7.** Binding of IC to THP-1 cells does not promote loss of CR2 from naive (Na) Raji cells, but does inhibit loss of CR2 from Raji cells opsonized with HB135. The overlap of the ○, △, and □ indicates that naive Raji cells alone as well as naive Raji cells incubated with either naive THP-1 cells or IC-containing THP-1 cells had the same signal at 30 min. However, after 30 min, 74% of CR2 was removed from the Raji cells opsonized with mAb HB135 and incubated with THP-1 cells; 47% was lost from the HB135-opsonized Raji cells when IC were prebound to the THP-1 cells.

### Table III. Binding of A1488 F(ab'){	extsubscript{2}}-anti-CR2 IC to THP-1 cells does not require C

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean Fluorescence Intensity, Channel No.</th>
</tr>
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<tbody>
<tr>
<td>A1488 F(ab'){	extsubscript{2}} IC</td>
<td>A1488 F(ab'){	extsubscript{2}} IC</td>
</tr>
<tr>
<td>Serum</td>
<td>337 ± 20</td>
</tr>
<tr>
<td>Medium</td>
<td>538 ± 9</td>
</tr>
<tr>
<td>Serum/EDTA</td>
<td>94 ± 8</td>
</tr>
</tbody>
</table>

*Average ± SD, n = 2, representative of two similar experiments.*
addition, Al633 rabbit anti-mouse IgG only bound to B cells after pretreatment with mAb HB135. We also found that typically ~70% of monkey B cells (identified as CD20 and CD45 positive) were CR2 positive (see below). We performed a study on a monkey that had a low titer of circulating anti-mouse IgG, due to an i.v. infusion of mouse IgG 1 year earlier. Al488 HB135 was infused i.v. as a bolus at a dose of 50 μg/kg. The Al488 HB135 bound rapidly (within 2 min) to circulating B cells (Fig. 8, A and C), and a fraction of cell-bound mAb was cleared within 30 min. ELISA measurements confirmed a low level of anti-mouse IgG in the preinfusion samples, which was eliminated 30 min after infusion of Al488 mAb HB135 (data not shown).

Rabbit anti-mouse IgG (100 μg/kg) was infused at the 60-min mark (Fig. 8, second arrow), and it immediately bound to the mouse mAb already on the B cells, as demonstrated by an increase in the Al633 goat anti-rabbit signal (MESF = 1520, 2 min after infusion; background 450 before infusion). Shortly after this second infusion, a fraction of the CR2-positive B cells (defined by either Al488 HB135 or the APFcy anti-CD21 probe) was temporarily removed from the bloodstream (Fig. 8, A and B), and the remaining CR2-positive B cells underwent a rapid loss of both bound Al488 mAb HB135 as well as the CD21 epitope (Fig. 8, C and D). At the 24-h point, the fraction of CR2-positive B cells found in the bloodstream returned to the preinfusion values (Fig. 8, A and B); however, the majority of the B cell-bound mAb HB135 demonstrable at 60 min had been removed from the cells (Fig. 8C), coincident with the loss of the majority of the CD21 epitope (Fig. 8D). In addition, the rabbit IgG bound to the B cells at 60 min was also cleared (final MESF = 445). Both spleen and liver sections were examined for localized Al488 mAb HB135 and rabbit IgG, and both proteins were clearly demonstrable and colocalized in the spleen (Fig. 9), but were not detectable in the liver (data not shown). We performed a similar experiment (but only out to 3 h) on another monkey and, we observed the same patterns of B cell binding, sequestration, and clearance of bound ligands and CR2 (data not shown).

Discussion

Comparison of E CR1 and B cell CR2 in the transfer reaction

The experiments reported in this work provide evidence that extends the E CR1 transfer reaction to B cell CR2. We find that C3dg-opsonic IC bound to Raji cell CR2 are transferred to acceptor macrophages in a reaction that is quite similar to the transfer reaction for C3b-opsonized IC bound to E (Figs. 1 and 2). These findings indicate that the kinetics of the two transfer processes are comparable. These results, along with our previous studies (12–14), indicate that in each case the transfer reaction follows a concerted process in which the C3 fragment-opsonized IC and donor cell C receptor are removed at the same rate. Moreover, CR2 removed from Raji cells is taken up by acceptor THP-1 cells, which again suggests that CR2 and its IC ligand are transferred together.

In analogy to the E model in which substrates are bound to E via CR1-specific mAbs, we investigated B cell CR2 opsonization with an anti-CR2 mAb (Figs. 3–7). In this model, the anti-CR2 mAb HB135 is used as a surrogate for C3dg-opsonized IC; that is, the mAb IgG is noncovalently associated with the B cell CR2 in place of the C3dg-Ag-IgG IC. Robust and stable binding of both these substrates to Raji cell CR2 is achieved after a brief incubation. Although the kinetics and affinity of binding of these substrates to B cell CR2 may be different, we suggest that the model allows analysis of the transfer reaction in the absence of C.

Substrates constructed with the anti-CR2 mAb HB135 are also transferred to THP-1 cells from Raji cells, and the kinetics of the transfer process as well as the concerted properties of the reaction are quite similar to those observed in the experiments with C opsonization. That is, mAb HB135 by itself, or in combination with
rabbit anti-mouse IgG, is taken up by THP-1 cells in a process that leads to loss of CR2 from Raji cells along with its appearance on THP-1 cells. The fact that the signal on the THP-1 cells due to extrinsic probes (e.g., APHcy anti-CR2 or Al633 anti-mouse IgG) decreases at longer transfer times suggests that a fraction of substrates taken up by the THP-1 cells is internalized after transfer. The signal on the THP-1 cells due to intrinsic IC labels reaches a maximum and does not decrease, indicating that the results cannot be explained by release of transferred ligands at later times.

The E transfer reaction requires Fc recognition of bound IC (12, 13, 35), and studies of the process with Raji cells suggest a similar requirement (Fig. 6). Although mAb HB135 is a suitable ligand for transfer, its F(ab)2 fragments are not transferred; moreover, when THP-1 cells are incubated with human IgG (to block FcR), transfer of mAb HB135 is abrogated. It is important to note that under these two conditions, in which the ligand bound to the Raji cells is not taken up by THP-1 cells, CR2 also remains on the Raji cells. That is, when the Fc component is not present or the IC substrate cannot effectively engage the FcR on the acceptor cell (Fig. 6), CR2 transfer is eliminated. Use of prebound IC to engage FcR on the THP-1 cells also partially inhibited transfer of substrates and loss of CR2 from Raji cells (Fig. 7). Finally, we find no evidence that IC binding to the THP-1 cells can activate these cells to promote direct loss of CR2 when these cells are incubated with naive Raji cells (Fig. 7). As in the E transfer reaction (12), it is likely, but not definitively proven, that the transfer reaction is initiated by recognition of Raji cell-bound IC by FcR on the macrophage, allowing close juxtaposition of the cells. This binding step is followed by proteolysis of CR2, thus allowing uptake of released IC and associated CR2 by THP-1 cells. The identity of the protease(s), presumably associated with the THP-1 cells, which may cleave E CR1 or B cell CR2, remains to be defined. Whether the transfer reaction and cleavage and release of cell surface proteins are most specific and/or unique to C receptors or perhaps include other proteins that express the SCR (38) remains to be determined. However, ligation of an unrelated cell surface protein, CD45, by an IgG2a mAb does not lead to loss of this protein in the transfer reaction (Fig. 5). Raji and THP-1 cells both express high levels of CD45, and the modest loss of anti-CD45 mAb from the Raji cells may be due to simple reequilibration, but could not have been mediated by the transfer reaction, as CD45 was not lost from the Raji cells.

Implications of the monkey model

Our studies of the B cell CR2 transfer reaction in the monkey model (Figs. 8 and 9) display some similarities and two notable differences compared with the primate E CR1 reaction: temporary B cell sequestration, and spleen localization. The results clearly indicate that Al488 mAb HB135 binds to monkey B cells in vivo. The early events seen in the first 30 min of the experiment are most likely caused by the low level of circulating anti-mouse IgG present in this monkey’s circulation. However, when the rabbit anti-mouse IgG was infused at 60 min, thus forming IC in situ on B cell CR2, a large fraction of the monkey B cells containing the bound IC was temporarily removed from the circulation. By 24 h, the percentage of CR2-positive B cells in the circulation had returned to the original value, but the cells had far lower levels of CR2, and, in addition, the amount of B cell-bound Al488 mAb HB135 and rabbit IgG had decreased considerably. The fact that CR2 levels were so low at 24 h indicates that it is unlikely that B cells found in the circulation at that time were newly synthesized B cells. Rather, we suggest that while the B cells were sequestered (presumably in the spleen; see below), the transfer reaction occurred, and that Al488 mAb HB135, rabbit anti-mouse IgG, and CR2 were all removed from the B cells in a concerted reaction. Apparently, the reaction did not go to completion because lower, but measurable levels of B cell-bound Al488 mAb were demonstrable on the presumably released B cells at 24 h. The fact that both infused Al488 mAb HB135 and rabbit IgG could be found colocalized in the spleen is consistent with this hypothesis (Fig. 9). We found no evidence for localization of fluorescent material to the liver, suggesting that with respect to organ localization, handling of IC bound to B cell CR2 may be somewhat different from handling of IC bound to E CR1.

Possible extension to other acceptor cells

In view of the importance of C, and in particular CR2 and its ligand, C3dg, in the immune response (1–11, 39), the in vitro and in vivo demonstration of a robust and rapid transfer reaction and the localization of CR2-associated IC to the spleen in the monkey model may reflect an important and natural process. Studies by several groups, initiated >30 years ago, revealed that IC infused into animals are either rapidly phagocytosed by macrophages in the spleen and/or liver, or taken up by resident, but not Ag-specific, B cells in the spleen and then later transferred to FDC (40–46). Although the mechanism for transfer to FDC was never clearly elucidated, it requires an intact C system and it is believed to play a key role in the immune response. Our in vitro studies demonstrate that transfer can occur between donor Raji cells and acceptor macrophages, in a reaction most likely mediated by FcR. The identity of other cells that may remove IC and associated B cell CR2 remains to be defined, but FDC, which have high levels of FcγRII as well as CR2, are likely candidates (4, 6, 7). As stated by Humphrey (44): “Why and how B lymphocytes with CR1 and CR2 receptors should transfer immune complexes containing complement to FDC, which are even more rich in CR1 and CR2 receptors, remains a mystery.” We suggest that close association of B cells, containing C3dg-opsonized IC, with FDC can be mediated by both FcγRII and CR2 on the FDC. This association may then allow transfer of the IC to the FDC for future presentation of intact Ag to specific B cells.

It has been reported by several groups that B cell-associated HIV, apparently bound to B cell CR2 as C3dg-labeled IC, is particularly infectious for T cells (19, 21, 47). Levels of B cell-associated CR2 are reduced in AIDS (19–21), and T cells appear to have the resident surface protease that can facilitate constitutive proteolysis of T cell-associated CR2 (24). Thus, it is reasonable that because of GP120–CD4 interaction, juxtaposition of T cells with C3dg-opsonized HIV IC on B cells can promote a transfer reaction leading to B cell CR2 cleavage and HIV uptake by and productive infection of the T cells. Although B cell CR2 is reduced in AIDS, a recent report suggests that FDC CR2 is in fact stable in this disease (47). Studies on another class of SCR-containing proteins, the selectins, have demonstrated rigorous requirements for proteolytic release of these proteins from leukocytes (38). Thus, we speculate that because of one extra SCR (48), FDC CR2 is not as easily proteolyzed as B cell CR2. Therefore, FDC CR2 may provide a stable site for localization and retention of intact Ag.

The studies of mouse CR2 initiated by Kinoshita and colleagues (27, 49, 50) have demonstrated that infusion of an anti-CR2 mAb in the mouse leads to loss of splenic B cell CR2. Although the mechanism that leads to this CR2 loss has not been clarified, we suggest it reflects the B cell transfer reaction in the mouse. Our in vitro studies, as well as our findings in the primate model, provide substantial evidence supporting the basic tenets of the B cell transfer reaction. Future investigations, based on the use of anti-CR2 mAbs in FcγR- and C-deficient mice (51), should lead to a more
detailed understanding of the role of C in enhancing the immune response to Ags in IC.

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