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Measles Virus Infection Synergizes with IL-4 in IgE Class Switching

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Increasing evidence suggests that viral infections are associated with the induction and exacerbation of asthma. One characteristic of human asthma is an increase in the levels of circulating IgE. Previous studies have shown that circulating IgE levels are elevated during the early phase of infection with measles virus (MV). We have shown previously that one mechanism by which viral infections can increase IgE levels is via an induction of IgE class switching through the activation of the antiviral protein kinase (dsRNA-activated protein kinase), leading to the activation of multiple NF-κB complexes. Therefore, to determine whether infection with MV can also induce IgE class switching, we infected the human Ramos B cell line with the Edmonston strain of MV. Infecting Ramos cells with MV did not result directly in either the activation of dsRNA-activated protein kinase or IgE class switching. However, a synergistic effect on IgE class switching was observed when Ramos cells were infected with MV before IL-4 treatment. Ab cross-linking of the MV receptor, CD46, mimicked the effects of MV infection in synergizing with IL-4 to induce IgE class switching, suggesting that viral hemagglutinin is involved in this synergistic effect. These data provide the first indication of a potential mechanism for MV-induced IgE up-regulation and suggest a model for a viral-induced exacerbation of IgE-mediated disorders such as asthma. The Journal of Immunology, 1999, 162: 1597–1602.

Allergic diseases are characterized by elevated levels of IgE Abs (1–3). Initially, resting B lymphocytes express IgM and IgD. Upon differentiation, B cells can express various Ig isotypes. Ig switching is regulated by secreted cytokines. During Ag presentation, T lymphocytes secrete various cytokines. In contrast to IFN-γ, which induces human B cells to switch to produce IgG subtypes (except for IgG4), the presence of IL-4 and IL-13 induces the production of IgE and IgG4 (4, 5).

An early step in IgE class switching is the transcription of an immature RNA from the Cε region of the Ig gene (6). This transcript, termed germline ε (Ge), is induced by IL-4 and to a lesser extent by IL-13 and cannot be translated into mature IgE protein. However, the presence of this transcript is essential for final switch recombination events leading to the production of the mature IgE transcript (8). Therefore, the expression of the Ge transcript is generally accepted as the first step of IgE class switching.

In addition to cytokines, the physical contact between T and B cells provides a second signal (costimulatory signal) that is necessary for Ig class switching. This second signal, which is provided by surface molecules such as CD40 and CD40 ligand (CD40L) (glycoprotein 39), is required for the activation of B cells (9).

Several other surface molecules, such as CD28/B7 and ICAM-1/LFA-1 participate in T and B cell interaction and Ig class switching (10, 11). Also, transmembrane-bound TNF-α, CD23/CD23 receptor, and LFA-3/CD2 reportedly cooperate with IL-4 for the production of IgE (12–14). Although the exact nature of the signals induced by the T and B cell contact is not yet clear, it is known that this interaction is essential for Ig class switching to any isotype.

Although it is commonly accepted that viral infections lead to increased Ab responses that are characterized by the IgE isotype, there is increasing evidence that infections with several viruses can lead to an expression of the IgE isotype (15–18). One of the viruses associated with a polyclonal increase in IgE expression is measles virus (MV) (18). We reported previously that viral activation of dsRNA-activated antiviral protein kinase (dsRNA-activated protein kinase [PKR], protein kinase dsRNA-activated) and subsequent activation of the NF-κB complex in human B cells leads to an induction of IgE class switching (19). Therefore, we determined whether MV infection can also induce IgE class switching through the activation of PKR. Infecting Ramos cells with MV alone did not lead to the activation of PKR or to the induction of Ge, suggesting that an alternative MV-induced mechanism may be responsible for the IgE up-regulation. We now report that MV infection acts synergistically with IL-4 to increase IgE class switching, and that this effect can be mimicked by cross-linking of the MV receptor CD46.

Materials and Methods

Cell line, culture conditions, and reagents

The human Burkitt’s lymphoma B cell line Ramos 2G6.4C10 was purchased from American Type Culture Collection (Manassas, VA). Cells (1 × 10⁵–10⁶/ml) were grown in RPMI 1640 medium supplemented with 10% FCS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, and gentamicin sulfate at 5 μg/ml at 37°C in a 5% CO₂ humidified chamber. The synthetic dsRNA polyinosinic:polycytidylic (poly(I.C)) was obtained from Sigma (St. Louis, MO); all other reagents were the highest quality available.

IFN treatment and in vitro kinase reactions

Ramos cells were treated with 100 U/ml of human IFN-α (Lee Biomolecular, San Diego, CA). After 24 h, cells were washed twice with isotonic...
buffer containing 20 mM HEPES (pH 7.5), 120 mM KCl, 5 mM MgOAc, and 1 mM DTT. Cells were then lysed in buffer containing 20 mM HEPES, 120 mM KCl, 5 mM MgOAc, 1 mM benzamide, 1 mM DTT, and 1% Nonidet P-40.

Reactions were performed as described previously (20). Briefly, mixtures for in vitro phosphorylation of cellular extracts contained 20 mM HEPES (pH 7.5), 90 mM KCl, 5 mM MgOAc, 1 mM DTT, 100 μM [γ-32P]ATP (specific activity 1 Ci/mM) (Amersham, Arlington Heights, IL), 100 μM ATP (Sigma), and equal amounts of detergent extract prepared from 1 × 10^6 cells in a final volume of 25 μl. dsRNA (poly(I:C)) was added to the reaction mixtures at 1 μg/ml following incubation at 30°C. After 10 min, the reactions were quenched by adding an equal volume of 2× SDS sample buffer containing 25 μl Tris-HCl (pH 6.8), 50 mM glycerol, 2% SDS, 0.0125% bromophenol blue, and 5% 2-ME. After boiling for 2 min, the reduced, denatured proteins were then subjected to electrophoresis through 10% SDS-PAGE. The labeled proteins were visualized by autoradiography of the dried gels.

**IL-4 treatment, viral infections, and Ab treatment**

Ramos cells were treated with 5 ng/ml of human rIL-4 (Sigma). The Edmonston wild-type strain of MV at multiplicity of infection (MOI) of 2.5 plaque-forming units (PFU)/cell was used for infections of Ramos cells. Mouse anti-CD46 mAb (GB24) was kindly provided by Dr. Christopher L. Karp (Johns Hopkins University) and was originally obtained from Dr. John P. Atkinson (Washington University, St. Louis, MO); the mAb was used at the indicated concentrations. At 48 h after infection or Ab treatment, cells were harvested and total cellular RNA was extracted.

**RNA extraction, detection of Ge, and quantitative analysis**

RNA was isolated using the TRIZol total RNA isolation reagent (Life Technologies, Gaithersburg, MD). After reverse transcription, the cDNA was amplified in the presence of 2 μg/ml of primers, 100 μM dNTPs, 0.25 U of Taq polymerase (Perkin-Elmer, Foster City, CA), 10 nM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, and 0.001% gelatin in a final volume of 25 μl. Primers for the Ge exon–intron sequence (5'-AGGGCTCCACTGCCCGGCACAGAAAT-3' and the Ge exon–derived sequence (5'-AGGGCTCCACTGCCCGGCACAGAAAT-3') were described by Gauchat et al. (21) in the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer (5'-CACAGTCCAGCCTACATG-3') and reverse primer (5'-TACCTTTGGAGCCATGTG-3') were used in the PCR reactions. PCR was performed in a DNA Thermocycler (Perkin-Elmer) for 42 cycles for Ge and for 25 cycles for GAPDH. For restriction endonuclease mapping, the 210-bp PCR product corresponding to Ge cDNA was purified using the QIAquick gel extraction kit (Qiagen, Chatsworth, CA). The purified fragment was digested with BglII enzyme (Life Technologies) for 2 h at 37°C, and the products were resolved on a 1% agarose gel using 10 μg/ml of poly dI.C:dI.C, 0.05% Nonidet P-40, 50 μg/ml of primers, 100 μM MgOAc, and 1% Triton X-100.

**Electrophoretic mobility shift assays (EMSAs)**

Cell extracts for EMSAs were prepared according to Schreiber et al. (22). EMSAs were performed using a [γ-32P]-end-labeled NF-κB (from κ light chain) consensus oligonucleotide (Promega, Madison, WI) and a custom synthesized oligonucleotide corresponding to CAAAT/enhancer-binding protein (C/EBP) and the STAT-6 response element of IgE germline promoter (5'-CGCTGTTGCTCAATGCTACATCTCCGAGA ACA-3') as described previously (23). The reactions (20 μl) consist of 2 μl of nuclear extract in buffer containing 20 mM HEPES (pH 7.5), 50 mM KCl, 0.2 mM EDTA, 10% glycerol, 40 μg/ml poly dI.C:dI.C, 0.05% Nonidet P-40 (which was omitted for C/EBP-STAT-6 assays), and 0.5 μl of labeled probe. After 30 min of incubation at 37°C, the protein/DNA complexes were resolved on 4.5% non-denaturing polyacrylamide gel and visualized by autoradiography of the dried gels.

**Results**

**MV infection does not activate PKR**

To determine whether MV-induced IgE up-regulation was due to the activation of PKR, Ramos cells infected with MV at an MOI of 2.5 PFU/cell. After 24 h, cells were harvested and detergent extracts were prepared. In vitro kinase reactions were performed in the presence or absence of 1 μg/ml of poly(I:C), which is a concentration of dsRNA previously determined to be optimal for PKR activation in Ramos cells (19). The data revealed that infecting Ramos cells with MV did not induce or activate PKR. However, treating cells with IFN-α did result in an induction of PKR that was activated in the presence of dsRNA (Fig. 1A).

To verify that MV could enter and replicate in Ramos cells, a kinetic study was performed using RT-PCR to detect MV HA mRNA. The results showed a time-dependent increase in the expression of HA mRNA in MV-infected cells (Fig. 1B). This finding is in agreement with previous studies showing that human B cells can support MV replication (24).

**MV synergizes with IL-4 in IgE class switching**

Our data showed that PKR was not activated by MV infection. To determine whether MV infection could, nevertheless, induce IgE class switching in human B cells by an alternative mechanism, Ramos cells were infected with the Edmonston strain of MV at an MOI of 2.5 PFU/cell. At 48 h postinfection, cells were harvested and total cellular RNA was extracted. RT-PCR was performed using primers specific to C/EBP-STAT-6. Consistent with our data showing that MV infection does not activate PKR, infecting the B cells with MV alone also did not result in IgE class switching. Treating the cells with IL-4, a potent inducer of IgE class switching, resulted in an

![Figure 1](http://www.jimmunol.org/)
induction of IgE class switching, as evidenced by the expression of Ge (Fig. 2A). The identity of Ge was confirmed by BglI digestion of the PCR product (data not shown).

In our preliminary experiments, infection with MV induced homotypic aggregation of the Ramos cells (data not shown). Because aggregation is postulated to be induced by other IgE class-switching costimulatory signals, such as anti-CD40 treatment, we hypothesized that the virus-induced cell to cell contact may also provide a costimulatory signal. To test this possibility, Ramos cells were infected with MV at an MOI of 2.5 PFU/cell and subsequently treated with 5 ng/ml of IL-4. Cells were harvested after 48 h, and the expression of Ge was determined by RT-PCR. Treating MV-infected cells with IL-4 resulted in a significant increase (~11-fold) in Ge expression over IL-4 treatment alone. However, as noted above, MV infection alone did not result in an induction of Ge (Fig. 2A).

Because MV infection did not directly induce or activate PKR, we determined whether an alternative mechanism was involved in this synergistic effect. It is known that CD46 is the receptor for MV and that viral HA mediates the initial virus/cell interaction (25). It is also known that Ab-mediated ligation of CD46 results in some biological events that are similar to those induced by MV infection (26). Therefore, we determined whether ligation of CD46 would provide a costimulatory signal for IgE class switching. Ramos cells were treated with 5 ng/ml of IL-4 and subsequently treated with anti-CD46 Ab. After 48 h, the expression of Ge was determined by RT-PCR. The data revealed that the addition anti-CD46 Ab to IL-4-treated Ramos cells induced a significant increase (~5.5-fold) in the expression of Ge (Fig. 2C). Therefore, the effect of anti-CD46 Ab treatment was similar to those observed with MV infection. It is interesting to note that the anti-CD46-induced IgE class switching costimulatory effect was less evident at 2 μg/ml than at 1 μg/ml. This may be due to an increase in the monovalent association of Ig molecules at higher concentrations.

These data suggest that the synergistic effect induced by MV infection may be mediated by ligation of CD46 by viral HA.

Activation of NF by IL-4 treatment, MV infection, and anti-CD46 treatment

Genetic knockout studies as well as Ge promoter studies have shown that NF-κB complexes and STAT-6 are involved in IgE class switching (27, 28). Furthermore, the cooperation between NF-κB and STAT-6 binding sites in IgE up-regulation has been documented (29). To determine whether IL-4 could activate STAT-6 in Ramos cells, we treated the cells with 5 ng/ml of IL-4; at different times posttreatment, the cells were harvested and extracts were prepared. Data from EMSAs showed that a STAT-6-specific complex was induced within 3 min after IL-4 treatment (Fig. 3A). The identity of the polypeptide in the complex was determined by competition assays using STAT-6- and C/EBP-specific probes (Fig. 3B).

To examine the effects of MV infection on NF-κB activation in Ramos cells, Ramos cells were infected with MV at an MOI of 2.5; whole cell extracts were prepared after indicated times posttreatment. Data from EMSAs showed that NF-κB was activated upon MV infection (Fig. 3C). The maximal level of NF-κB activation was observed at 24 h posttreatment. To determine whether anti-CD46 treatment could also activate NF-κB, we treated the cells with 1 μg/ml of anti-CD46 Ab. The data revealed that, similar to MV infection, treating Ramos cells with anti-CD46 resulted in a time-dependent activation of the NF-κB complex (Fig. 3D). However, infection with MV or treatment with anti-CD46 did not result in STAT-6 activation in Ramos cells (data not shown).

FIGURE 2. MV and IL-4 synergize in IgE class switching. A, To determine whether MV infection could act as a costimulatory signal in IgE class switching, Ramos cells were either mock-treated, treated with 5 ng/ml of IL-4 alone, infected with the Edmonston wild-type strain of MV at an MOI of 2.5 PFU/cell, or infected with MV and subsequently treated with 5 ng/ml of IL-4. After 48 h, cells were harvested and total cellular RNA was prepared. The expression of Ge was determined by RT-PCR using specific primers. The housekeeping gene GAPDH was amplified as an internal control. Results are representative of four separate experiments. B. The relative increase in the expression of Ge presented in A was determined by quantitative analysis of the densitometrically scanned gel. C. To determine whether the observed synergistic effect was mediated by CD46, Ramos cells were either mock-treated (column A), treated with 5 ng/ml of IL-4 alone (column B), treated with 1 μg/ml of anti-CD46 alone (C), treated with 2 μg/ml of anti-CD46 alone (column D), treated with 5 ng/ml of IL-4 and anti-CD46 at 1 μg/ml (column E), or treated with 5 ng/ml of IL-4 and anti-CD46 at 2 μg/ml (column F). The relative increase in Ge expression was determined by quantitative analysis after densitometric scanning of the gel. Error bars represent the SD of the mean between three separate experiments.
Discussion

It is known that higher circulating IgE levels are associated with MV infections; however, the mechanism of this up-regulation is not known. We recently reported that activation of PKR by viral infection results in an induction of IgE class switching in human B cells (19). The activation of PKR is dependent upon the presence of dsRNA that is not detectable during the normal life cycle of eukaryotic cells; however, this dsRNA is present during the life cycle of many viral strains. Because infecting human B cells with MV did not result in PKR activation or IgE class switching, direct PKR activation does not appear to be involved in MV-induced IgE up-regulation. However, our data showed that IL-4 treatment and infection with MV had a synergistic effect on IgE class switching.

The interaction of several cell surface molecules such as CD40/CD40L, LFA-1/ICAM-1, and CD23 may be necessary for efficient IgE class switching (9–11). However, using flow cytometry experiments, our data revealed that MV infection of Ramos cells did not result in an increase in the surface expression of LFA-1.
ICAM-1, or CD23 (data not shown). It is known that adhesion molecules may be activated without any detectable increase in surface expression; therefore, our data suggest but do not prove that up-regulation of these adhesion molecules is not involved in the MV-induced synergistic effect. It is also known that the stimulatory signal exerted by CD40/CD40L interaction coincides with homotypic aggregation of cells (30). Because our initial experiments showed that infecting Ramos cells with MV induced homotypic aggregation, we hypothesized that MV infections may provide a costimulatory signal for IgE class switching through other surface molecules.

MV gains entry into cells by attaching viral HA to its putative receptor CD46 (membrane cofactor protein) (25). This cell surface molecule is a member of a family of regulators of complement activation, and Ab cross-linking of CD46 has been shown to result in biological effects similar to MV infection (25, 31). Therefore, we determined whether MV-induced CD46 cross-linking was the mechanism of the observed synergistic effect. Cross-linking experiments revealed that Abs to CD46 mimicked the effects of MV infection, suggesting a molecular pathway for MV-induced IgE up-regulation. It is important to note that the synergistic effect observed by IL-4 and MV infection was consistently greater than that observed by IL-4 and anti-CD46. The exact reason for this difference is not clear; however, it is possible that in contrast to anti-CD46 Ab, which provides dimeric cross-links, the presence of MV HA protein on the surface of infected cells provides multi-meric cross-linking of CD46 molecules. It is important to note that previous in vivo studies have shown that monocytes are the major MV-infected cell population (32). Therefore, during in vivo MV infections, the IgE class switching costimulatory effect may be provided by the interaction of infected monocytes with B cells. Furthermore, it has recently been reported that, in addition to CD46, another cell surface molecule may be used by MV for cell entry (33), suggesting that the cross-linking of other surface molecules may contribute to this synergistic effect.

It is also known that MV infection leads to an elaboration of IL-4 (33). IL-4 is a potent inducer of IgE class switching; however, the increase in IgE that is seen during MV infection is probably not due to an elaboration of IL-4, because IL-4 was induced in vivo as a secondary response to MV infection (34). Furthermore, in our in vitro assays, MV infection alone did not result in IgE class switching, nor did it result in expression of IL-4 mRNA (data not shown).

Promoter studies on the transcriptional events leading to IgE expression have shown that activation of both NF-κB and STAT-6 molecules is necessary for the efficient induction of IgE class switching, as detected by expression of Ge (27, 28). The data from our EMSA experiments showed that STAT-6 was activated within 3 min after IL-4 treatment of Ramos cells, and that infecting Ramos cells with MV induced activation of the NF-κB complex. Therefore, we believe that the observed synergistic effect is mediated by activation of the two necessary IgE regulatory NF, namely STAT-6 and NF-κB (29). However, at the present time, we cannot exclude the involvement of other NF that are involved in IgE class switching, such as B cell-specific activating protein and C/EBP (35, 36).

In addition to infection with MV, IgE levels are elevated in infections with respiratory syncytial virus, influenza virus, and members of picornaviruses and flaviviruses (15–18, 37, 38). Based on our previous data, we believe that viral infections that lead to the activation of PKR can directly induce IgE class switching and consequently increase the risk of primary induction of IgE-mediated disorders such as asthma and allergy. However, infections with viral strains that can cross-link relevant B cell surface molecules and synergize with IL-4 may also lead to exacerbation of IgE-mediated disorders. If so, viral infections that can activate PKR and cross-link costimulatory surface receptors will provide the strongest signal for the induction and exacerbation of IgE-mediated disorders. Further studies are needed to test this concept.

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